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FIRST LECTURE.

THE EVOLUTION OF THE SPOROPHYTE IN THE HIGHER PLANTS.

DOUGLAS HOUGHTON CAMPBELL.

The questions relating to the origin of organic structures must always possess great interest for the biologist, and when I was asked to speak before the students at Woods Holl, it seemed to me that a discussion of recent views bearing on the development of the spore-producing structures of the higher plants, *i.e.*, the Archegoniates and seed-bearing plants, would not be inappropriate.

There is good reason to suppose that among plants, as among animals, the most primitive forms were aquatic; and it is highly probable that many existing fresh-water algæ are but slightly modified descendants of these ancestral types. This is evinced by the great uniformity shown by existing green algæ all over the world. Most genera and many species are cosmopolitan, and they exhibit far less variety than is shown by their larger and more specialized marine relations. Presumably the conditions in fresh water have changed but little, and as the more specialized forms have taken to the land, or have developed in the sea, the few that remained in their primitive environment have been subjected to much less competition, and have probably persisted with but little change from very remote times.

Leaving aside certain forms of doubtful affinities, like the bacteria and blue-green algæ, the existing forms which represent most nearly the ancestors of the higher plants are the Volvocineæ and Protococcaceæ. The former are, as all botanists know, free-swimming green organisms which show resemblances on the one hand to low animals, and on the other are very like the free-swimming reproductive cells, or zoöspores, of many of the higher algæ. The latter probably originated from forms like the simpler Volvocineæ by the loss of motility associated with the development of a continuous cellulose membrane about the vegetative cells. This stage in the evolution of the green algæ is represented by some of the simpler Protococcaceæ. Above these unicellular forms are a number of filamentous plants, single rows of nearly uniform cells.. Such plants are Œdogonium or Conferva, which well represent the next step in the evolution of the plant body.

The differentiation of the reproductive cells in the algæ, while it offers one of the most interesting and instructive examples of the evolution of plant structures, must be passed over here. It may be mentioned, however, that the differentiation of the sexual cells has evidently taken place quite independently in several groups of algæ.

In the further development of plant types two very important factors are to be considered. First, the adaptation to a marine existence, and, second, the exchange of an aquatic for a terrestrial life.

The conditions in the ocean are markedly different from those in fresh water, and most plants which have adapted themselves to life in the sea have become decidedly changed. The salinity of the water has no doubt been one of the factors in these changes, but more important is probably the question of light. The two most characteristic groups of sea plants, the red and brown seaweeds, are provided with special pigments in addition to the chlorophyll, and there is little question that these pigments are developed, in part at least, in response to changed light conditions.

As the conditions of light and temperature in a marine environment are far more constant than those in fresh water, we find, as a rule, that marine algæ, especially those inhabiting the deeper water, are more susceptible to changes of light and temperature than are most fresh-water forms.

While certain seaweeds growing between tide-marks are subject to exposure to the air, it is only for a short time, and we find in most such algæ a development of mucilaginous tissue which prevents their complete desiccation.

These seaweeds have adapted themselves perfectly to their peculiar environment, and such highly specialized forms as the great kelps and many red algæ probably represent the highest types of these marine plants. They have diverged widely from the simpler fresh-water algæ, and there is no reason to suppose that they have given rise to any higher types.

The simple fresh-water green algæ, which, so far as we know, most nearly represent the ancestors of the terrestrial green plants, differ much in their conditions of life from the seaweeds. Most bodies of fresh water are subject to great fluctuations of depth, often drying up completely for long periods, or sometimes being frozen. It is obvious that plants living under such conditions must be very resistant, and we find that such is the case among most green algæ. Not only, as a rule, are they capable of enduring a great range of temperature, but usually at the end of their vegetative period they produce special cells, "spores," which can endure complete desiccation without injury, and are also uninjured by freezing. By means of these "resting-spores" the plant is carried over from one growing period to the next, and when the conditions are favorable, the spores germinate and give rise to a new generation. This production of resting-spores is one of the most striking differences between these fresh-water algæ and their red and brown relations in the sea, where there is usually no necessity for such resting-spores.

Certain green algæ, like some species of the common genus Vaucheria, may be considered amphibious, as they do not actually grow in the water, but are exposed to the air on the surface of moist earth, from which they absorb the water necessary for their growth. The ability to thus grow with a diminished water supply is an evident advantage, and in some such way as this it is probable that the first strictly terrestrial plants originated from some originally aquatic algal ancestors.

We can imagine some such forms gradually becoming better and better able to vegetate on the mud left by the subsidence of the water, and finally becoming adapted to life on land. The lower liverworts, which represent the simplest types of existing land plants, probably originated in some such way. There are still existing certain amphibious liverworts, species of Riccia, which live for the most part as floating aquatics, but sometimes settle down in the mud left by the subsiding water, or even creep up the muddy banks and establish themselves as land plants. This seems to be most commonly done before the development of the reproductive organs. It is very likely that the individual history of these liverworts illustrates the origin of the typical forms from their aquatic ancestors.

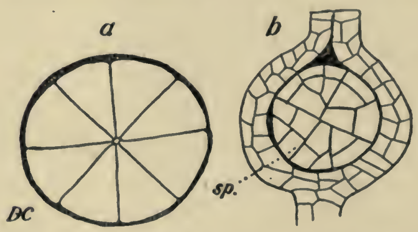

FIG. 1. — *a*, Diagram of a section through the germinated resting-spore of an alga (Coleochæte), showing the mass of cells within. Each cell of the rudimentary sporophyte produces a single zoöspore. *b*, Longitudinal section of the lower part of the fertilized archegonium of a liverwort (Riccia), showing the contained sporophyte, *sp.*, developed from the egg-cell. Each cell of the sporophyte, except the outside ones, gives rise later to four non-motile spores.

Among those algæ which approach most nearly to the lower liverworts there is a hint of the characteristic alternation of generations so conspicuous in the mosses and ferns. The egg-cell in such algæ, on being fertilized, produces a resting-spore which may be invested with a protective envelope of cells. This resting-spore (oöspore) does not, on germination, produce a new plant at once, but a globular cellular mass is formed (Fig. 1, *a*), each cell of which gives rise to a motile zoöspore, which then grows into a plant like the parent one.

This alternation of sexual and non-sexual plants becomes, as is well known, very prominent in the mosses and ferns, or Archegoniates as they are called on account of the peculiar female organ, the archegonium.

While there is unmistakable evidence of relationship between the lower liverworts and the green algæ, it must be admitted that at present the gap between the two groups is a very great one, and we are not in a position to state positively just where is the point of contact between Archegoniates and algæ.

The structure of the archegonium (Fig. 2) is very constant throughout the Archegoniates, but has very little in common with the much simpler oögonium, or female organ of the green algæ, and its origin is still a matter of conjecture.

Aside from the character of the sexual organs, there is no difficulty in homologizing the sexual plant (gametophyte) of the lower liverworts and that of certain green algæ.

As a result of the change from the aquatic to an aërial environment, we find the tissues of the gametophyte in the liverworts more resistant to loss of water than is the case in the algæ. The outer cell-walls are cuticularized, and there is more or less specialization of the inner tissues, and this specialization may be very pronounced, as we find in such large liverworts as Marchantia. Finally, in the leafy liverworts and true mosses, the gametophyte develops a definite axis and special assimilating organs, leaves.

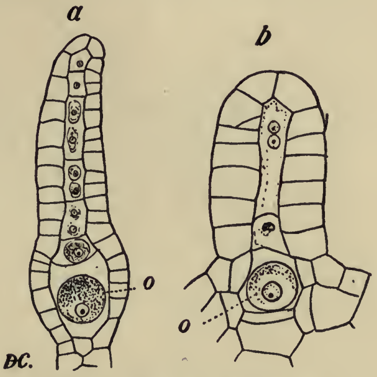

FIG. 2.—*a*, The archegonium, or female organ, of a liverwort (Targionia), seen in longitudinal section. *b*, A similar section of the archegonium of the cinnamon fern; *o*, the egg-cell.

It is the sporophyte, however, or non-sexual plant, developed from the fertilized egg within the archegonium, that we wish especially to consider. We have already intimated that in certain algæ the oöspore, on germination, gives rise to a globular cell-mass (Fig. 1, *a*), each cell of which produces a zoöspore. In the lowest liverworts, *e.g.*, Riccia (Fig. 1, *b*), a similar globular cell-mass is produced from the fertilized egg, but there is a much shorter period intervening between fertilization and the germination of the spore. This cell-mass, while directly comparable to the corresponding structure in Coleochæte, differs from it in two important particulars. In the first place, a single external layer of cells in the sporophyte of Riccia (Fig. 3, *a*) remains sterile, and, secondly, each cell of the interior sporogenous tissues, instead of producing a single zoöspore, gives rise to four non-motile, thick-walled spores, which do not germinate immediately, but are capable of becoming quite dry without losing their vitality, thus answering physiologically to the single oöspore of the algæ. In short, the resting period is here shortened, owing to the power of the gametophyte to grow on land, and for the protection of the growing sporophyte within the archegonium.

It must be remembered, however, that for fertilization to be effected, the gametophyte must be covered with water, or, as it were, go back to the original aquatic condition, since in all Archegoniates water is necessary for the opening of the sexual organs, as well as for conveying the ciliated spermatozoids to the archegonium.

This amphibious habit of the gametophyte is retained throughout the whole group of Archegoniates, and is even found in the lowest seed plants, *e.g.*, Cycas.

As the archegoniate type becomes better developed, we find a change taking place in the relative importance of gametophyte and sporophyte. While in certain groups like the leafy liverworts and the true mosses there is a considerable amount of specialization in the gametophyte, in other types, especially those which seem to lead up to the ferns, the gametophyte is much simpler in structure, and it is the sporophyte which becomes the conspicuous phase. We must remember, however, that in these forms the sexual organs, archegonium and antheridium, are essentially the same as in the lower liverworts and true mosses, and like them require water in order that fertilization may occur.

As every botanist knows, the fern spore on germination produces, not the fern as we ordinarily understand it, but the inconspicuous liverwort-like gametophyte, or "prothallium," which not only closely resembles in general appearance a simple liverwort, but also bears reproductive organs of very similar structure.

The change in the relative importance of gametophyte and sporophyte has evidently been a very gradual one and is well illustrated in the existing Archegoniates.

It is safe to assume that the most primitive Archegoniates had a sporophyte in which all the cells were sporogenous as they are in Coleochæte, but no such primitive forms are known to exist at present. The simplest known type of sporophyte is that of Riccia (Fig. 3, *a*), already referred to. Here, although a thin peripheral layer of cells is sterile, much the greater part of the sporophyte is composed of sporogenous tissue, all of whose cells produce spores.

There are still existing a number of low liverworts which very clearly show the mode of progression from the simple capsule filled with spores, found in Riccia, to the more highly organized sporophyte of the higher forms. The first step in the development of the sporophyte is the separation of the upper spore-bearing portion from a lower part, which becomes an organ of absorption, — the foot, — and thus the sporophyte begins to assume the character of an individual plant with both vegetative and reproductive tissues. This stage is well shown by the little liverwort Sphærocarpus (Fig. 3, *b*). Here the upper part of the sporophyte becomes differentiated into a globular capsule, with a well-developed wall enclosing the sporogenous tissue, some of whose cells remain undivided and serve to nourish the growing spores derived from the other sporogenous cells.

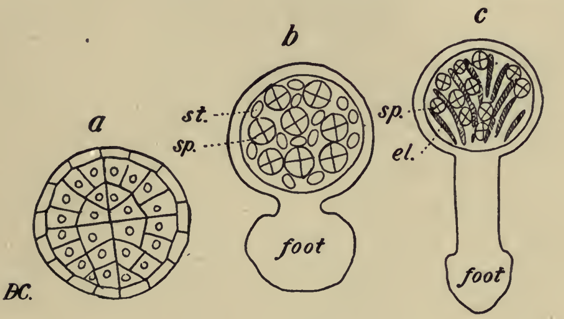

FIG. 3. — Diagram showing the evolution of the sporophyte in the typical liverworts. *a*, Riccia; *b*, Sphærocarpus; *c*, Porella. In Riccia all but the single outer layer of cells produce spores; in Sphærocarpus an absorbent organ or foot is present, and some of the sporogenous tissue remains sterile, forming the sterile cells, *st.*; in Porella a stalk is developed between the foot and the capsule, and the sterile cells within the capsule develop into elaters, *el.*; *sp.*, tetrads of spores.

In the higher liverworts (Fig. 3, *c*) the foot is well developed, and a stalk is usually formed between it and the capsule. In the latter the sterile cells form the curious spirally thickened elaters, whose principal function is to help in distributing the spores.

While the last-mentioned type of sporophyte is characteristic of most liverworts, there is one order — sometimes considered to be a class, intermediate between the liverworts and Pteridophytes, or ferns. In these Anthocerotaceæ, of which the genus Anthoceros is the most highly developed, the sporophyte becomes far better developed than in any of the typical liverworts, although the gametophyte is exceedingly simple. In these plants the sporophyte (Fig. 4, *a*) becomes relatively very large, and is remarkable for the subordination of spore production to the vegetative life of the sporophyte. The sporogenous tissue is reduced to a single layer of cells — at least in the beginning. Outside of this sporogenous layer are several layers of green cells with large air spaces between them, which communicate with the outside atmosphere by means of stomata precisely like those on the leaves of the higher plants. There is no question that the spongy green tissue forms a very efficient assimilative apparatus, like the green tissue of ordinary leaves, so that the sporophyte is probably quite able to provide its supply of carbon from the atmosphere. The axial strand of cells, the columella (*col.*), corresponds in position to the young vascular bundle in the fern sporophyte, and possibly may, like it, serve as a means of conducting water. No root is developed, however, and the sporophyte must get its supply of water from the gametophyte by means of the very large foot.

Unlike all other liverworts, the sporophyte in the Anthocerotaceæ develops a zone of growing cells between the foot and the upper part of the sporophyte; and these active cells keep adding new tissue to the base of the sporophyte, which may thus continue to grow for several months. Were the sporophyte connected with the earth by a root, it would be entirely able to take care of itself and be quite independent of the gametophyte.

In tracing the evolution of the sporophyte in the Bryophytes, or mosses, the most marked characteristic is the gradual reduction of the sporogenous tissue, and its subordination to the purely vegetative part of the sporophyte, which finally approaches an independent condition, most evident in such forms as Anthoceros and the true mosses. In these plants, by the development of a special assimilative green tissue, communicating with the outside atmosphere by means of stomata, the sporophyte becomes entirely independent, so far as its supply of carbon is concerned, as it can utilize the CO_2 of the atmosphere. It remains, however, to some degree dependent upon the gametophyte, from which it secures water and some nitrogenous food materials, very much as a parasitic plant does from its host.

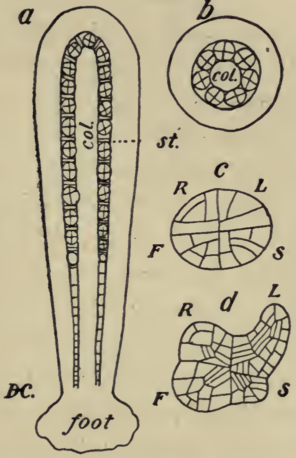

Fig. 4.—*a*, Diagram of a longitudinal section of the sporophyte of Anthoceros, the nearest approach to the sporophyte of the ferns found among the Bryophytes. The sporogenous tissue is confined to a single layer surrounding the central strand of tissue, or columella, *col.* A large foot is developed, and the outer tissues develop chlorophyll. The tetrads of spores are separated by sterile cells, *st.* *b*, Cross-section of the same. *c*, Diagram of a vertical section of a young fern embryo showing its close resemblance to the corresponding stage of the liverwort sporophyte. *d*, A similar section of an older fern sporophyte showing the formation of the external organs—stem, *s.*; leaf, *L.*; root, *R.*; and foot, *F.* Each of these young organs contains an axial strand of elongated cells, the beginning of the vascular bundle.

While the higher Bryophytes show considerable differentiation in the sporophyte, so far as the tissues are concerned, there is little or no external differentiation, and nothing comparable to the leaf and stem found in the sporophyte of the higher plants. If, however, in such a form as Anthoceros, we could imagine the large absorbent organ, the foot, to penetrate through the gametophyte into the earth, so that the sporophyte could obtain water and the mineral constituents directly from the earth, we should then have the sporophyte an entirely independent organism.

This completely independent sporophyte is first encountered in the ferns or Pteridophytes — "vascular cryptogams," as they are sometimes called. The simple liverwort-like gametophyte of the fern, as we have already stated, bears sexual organs of very much the same structure as those of the liverworts, and from the egg-cell within the archegonium is produced, as the result of fertilization, a sporophyte which in its early history follows very closely the development of the sporophyte of a liverwort (Fig. 4, *c*). As a result of the early divisions, a nearly globular mass of cells is formed, but very soon a difference is manifest. Instead of retaining the original globular form, as in Riccia, or simply forming a cylindrical body, as in Anthoceros, the young fern sporophyte very early develops several growing points (Fig. 4, *d*), which soon cause it to lose its original form; and it is evident that each of the growing points is the beginning of a special organ. One of these shows itself to be the cotyledon or first leaf, another becomes the apex of the stem, and a third develops into the primary root of the young sporophyte. This external differentiation of the sporophyte, which by virtue of the root becomes a self-supporting plant, at once sharply separates the ferns from even the highest mosses. This development of special organs is also associated with far more perfect tissues than are ever found in the mosses, the most noteworthy of these being the characteristic vascular bundles.

Of the primary organs, the leaf or cotyledon — at least in the true ferns — soon assumes a flattened form, and the green tissue of which it is mainly composed indicates that its principal function is that of carbon assimilation.

The root soon breaks through the tissues of the gametophyte and grows downward into the substratum, thus putting the young sporophyte into direct communication with the supply of water. As soon as the sporophyte became thus entirely self-supporting, a new type of plant was evolved which was destined to become the predominant type of the future. The development of a sporophyte with definite axis or stem, to which are attached leaves and roots, is the characteristic of all the higher plants, or the "vascular plants" as they are sometimes called.

In the ferns, spore production, which in the lower Archegoniates is the sole function of the sporophyte, becomes entirely subordinated to the vegetative existence of the sporophyte, which may reach a very large size, some of the tree ferns having stems ten metres or more in height, and living for many years. Spore formation in these forms does not take place until several years after the sporophyte is first formed. Finally, however, the spores are developed from a sporogenous tissue, exactly as in the liverworts and mosses. The spores are, however, borne in special organs—sporangia, which in the ordinary ferns are borne in groups on the back of the leaf. The form and position of the sporangia are quite constant and form one of the best means of classifying the Pteridophytes. The first hint of a segregation of the sporogenous cells is met with in Anthoceros and its allies, where the continuous layer of sporogenous tissue is imperfectly divided into fertile and sterile portions (Fig. 4, *a*). This sterilization of a part of the sporogenous tissue is probably the first step in the direction of the special spore-bearing organs, or sporangia, to which the spore formation is restricted in the Pteridophytes. The development of simple sporangia probably occurred very early in the history of the group.

With the growing importance of the sporophyte is associated the development of very perfect tissues of different kinds, most of which we already find present in the ferns. Of these, the vascular bundles already referred to are the most characteristic. It is not unlikely that the central strand of elongated cells, occupying the axis of the sporophyte in Anthoceros and in most of the true mosses, may be compared to a rudimentary vascular bundle, but none of the Bryophytes show the characteristic tracheary tissue, the woody part of the bundle in the higher plants.

In all the plants above the mosses it is the sporophyte which attracts our attention. We cannot here go into details as to the extraordinary development of the vegetative organs of the sporophyte, but must confine our attention to the evolution of the spore-bearing organs, or sporangia.

The gradual development of the sporangium is well illustrated in the true ferns, or Filicineæ, the predominant type, at present, of the Pteridophytes.

We have already referred to the beginning of a segregation of the sporogenous tissue in Anthoceros. The nearest approach to this condition in the Pteridophytes is found in the peculiar genus Ophioglossum (Fig. 5, *a*). Here the spore-bearing part of the leaf has the form of a spike with two rows of sporangia which are hardly indicated at the surface, but consist practically of large cavities filled with spores sunk below the surface of the leaf and opening at the surface by a transverse slit. It has even been claimed that here, as in Anthoceros, there is a continuous layer of potential sporogenous tissue which becomes divided into separate sporangia by the sterilization of portions situated at regular intervals. A very important difference between the sporangia of Ophioglossum and the sporogenous tissue of Anthoceros is that in the latter all the spores are discharged through a single opening at the apex of the sporophyte, while in Ophioglossum each group of spores has its own opening at the surface of the leaf.

Starting with the very simple type of sporangium found in Ophioglossum, we find a very perfect series of forms still existing, which lead up to the type found in the more specialized ferns. Thus within the genus Botrychium (Fig. 5, *c*, *d*), which is closely allied to Ophioglossum, there are several species which illustrate most beautifully almost every intermediate form. The simplest ones have a few large sporangia, more or less sunken in the tissue of the leaf, and closely resembling those of Ophioglossum ; while the highest members of the series have numerous small sporangia which are borne upon a short stalk or pedicel, and are very much like those of the ordinary or leptosporangiate ferns, whose sporangia are developed entirely from the epidermal tissue. The genus Osmunda, which includes the common cinnamon fern of the eastern United States, is in certain respects intermediate between the typical leptosporangiate ferns and the type found in Botrychium, although it must be admitted that the affinities of Osmunda are perhaps quite as evidently with another ancient group of ferns, the Marattiaceæ, an order of tropical ferns which are closely related to many extinct carboniferous ferns.

It is probable, although this cannot be absolutely proved, that such forms as Ophioglossum and Osmunda, which have the whole leaf, or leaf segment, covered with sporangia, are more primitive than those in which the sporangia are borne upon the backs of ordinary leaves.

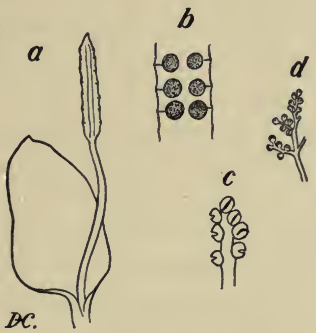

Fig. 5.—*a*, The upper part of a leaf of an adder-tongue fern (Ophioglossum), showing the spike of simple sporangia. *b*, Diagram of a longitudinal section of a part of the spike, showing the cavities containing the spores. *c*, Sporangial spike of *Botrychium simplex*, with the few large sessile sporangia. *d*, Part of the sporangial spike of *B. Virginianum*, with numerous small, somewhat stalked sporangia.

Wherever the sporangia are borne upon specially modified leaves (sporophylls) we have structures which may be legitimately compared to the flowers of the seed-bearing or "flowering" plants, where the flower consists essentially of similar modified spore-bearing leaves, or sporophylls.

In passing from the lower to the higher Pteridophytes, there is manifested in several places a remarkable phenomenon which is of the greatest importance as bearing upon the origin of the seed-bearing plants. This is known as "heterospory," or the production of two sorts of spores.

With the increasing importance of the sporophyte in the Pteridophytes, there is an accompanying reduction in the gametophyte. While the latter in the lower ferns is a comparatively large liverwort-like plant, sometimes living for several years, we find in the higher forms that it becomes much smaller and lives but a short time, and it is not uncommon to find that separate male and female plants are produced ; but there is no apparent difference in the spores from which they arise.

In the heterosporous Pteridophytes, however, some of the sporangia produce a small number of spores, sometimes but one. These "macrospores" are many times larger than the much more numerous "microspores" developed in the remaining sporangia. From the large spore is produced the female gametophyte, from the small one the male. These are both much reduced, and their whole development may take place within twenty-four hours. The development of the sporophyte from the fertilized egg is in such cases very rapid, and it soon becomes independent, and all trace of the gametophyte disappears. In most of these heterosporous forms the gametophyte never becomes self-supporting, depending for its growth upon the materials already accumulated in the ripe spore. Such gametophytes develop no chlorophyll, and scarcely exceed in bulk the spore from which they arise, and within which they are contained, there often being only sufficient splitting of the spore coat to expose the reproductive organs.

In the club mosses of the genus Selaginella, heterospory is carried still further. Before the spores are discharged from the sporangium they have already undergone the first steps in germination, and by the time the spore is ripe the gametophyte has begun to develop within it. In this case the growing gametophyte does not depend entirely upon the food stored in the spore, but draws its nourishment to some extent directly from the sporophyte through the wall of the sporangium, which thus serves not only to protect the spores but also, to some extent, as a medium for the transfer of food to the developing gametophyte. In short, the relation of gametophyte and sporophyte is the reverse of what obtains in the mosses, where it is the sporophyte which is permanently dependent on the gametophyte.

It is very certain that heterospory has developed quite independently in all the chief groups of Pteridophytes, and there is evidence from the fossil remains of these plants that several heterosporous types have become extinct. The multiple origin of this peculiar phenomenon makes it exceedingly probable that the formation of seeds, which is simply a further development of the same phenomenon, has also originated more than once; and it is highly improbable that all of the seed-bearing or flowering plants, to use a common term, have descended from a common stock. It is much more in accordance with the known facts to assume a multiple origin for the seed-bearing plants or Spermatophytes.

These highest of all plants do not possess any structures which are peculiar to them. The flowers consist of one or more sporophylls, or sporangial leaves, which are homologous with those of the ferns. Upon these sporophylls — stamens, carpels — are borne sporangia which are directly comparable to those of the heterosporous Pteridophytes. The microsporangia are here known as pollen sacs, and the macrosporangia as ovules. The pollen sacs and spores agree in their most minute particulars with the corresponding microsporangia and spores of the heterosporous Pteridophytes. The spores in both cases are shed, and the development of the very rudimentary gametophyte takes place away from the sporophyte. The pollen spores always arise in tetrads, like the spores of all Archegoniates, and the structure of the ripe pollen spore is not noticeably different from the spore of a liverwort or moss.

With the macrospores the case is somewhat different. The macrosporangium in the flowering plants differs in some respects from that of the heterosporous Pteridophytes. Within it is borne a single spore (embryo sac) which may be one of four sister-cells, but is not necessarily so. The macrospore never becomes free, but is retained permanently within the sporangium, where it germinates, usually while the sporangium is still attached to the sporophyte, but occasionally after it has become separated.

Within the macrospore, or embryo sac, is developed a rudimentary female gametophyte, closely resembling that of some of the heterosporous Pteridophytes, and in the lower forms, like the coniferous trees, archegonia are produced. The embryo sporophyte is formed from the fertilized egg-cell, as in the ferns, and is surrounded by the tissue of the gametophyte, here called the "endosperm." During the growth of the embryo the outer tissues of the sporangium become hard, and finally the sporangium with the enclosed spore and gametophyte falls away as a seed.

The permanent retention of the macrospore within the sporangium necessitates a somewhat different mode of fertilization, and we find, therefore, that the pollen spore, instead of developing an antheridium and motile spermatoids, produces a long tube into which the sperm-cells pass. The pollen tube grows through the tissues overlying the apex of the embryo sac, much as a parasitic fungus penetrates the tissues of the host plant. When it reaches the archegonium the sperm-cells are discharged, and one of them passes directly into the egg-cell.

One of the most important discoveries of recent years is the presence of large ciliated spermatozoids in the lowest types of seed-bearing plants; *e.g.*, Zamia, Cycas. In these the pollen tube becomes much distended with water, and finally bursts, discharging the large spermatozoids with the fluid into a space above the archegonium, which the spermatozoids enter just as they do in the ferns. This discovery removes the last barrier separating the ferns from the flowering plants, and there can be no further doubt as to the close relationship of these groups.

The Gymnosperms — Cycads, Coniferæ, etc. — are undoubtedly much nearer the ferns than they are to the ordinary flowering plants, or Angiosperms. It is usually taken for granted that the latter originated from the Gymnosperms, but it is quite as likely that they constitute an entirely independent developmental line originating directly from the ferns, which has proved itself better fitted on the whole to modern conditions than are the simpler and more ancient Gymnosperms, which they have largely superseded.

Although the sporophyte in some of the Gymnosperms, such as the giant conifers, is often of gigantic size, it never shows as complex structure as is found in most of the Angiosperms, or typical flowering plants. The latter represent the highest plant type, and the sporophyte exhibits almost endless variety, especially in the flowers. The gametophyte, however, is reduced to a microscopically small body consisting of a few cells only.

The type of structure exhibited by the Angiosperms has been remarkably successful, and the great bulk of existing terrestrial plants are Angiosperms, which have to a great extent replaced the ferns and Gymnosperms of earlier geologic times. In the water, however, where conditions have changed less, the lower types of plants still persist, and the Angiosperms are much less important.

SUMMARY.

In passing from the higher algæ and lower liverworts to the ferns and flowering plants, it is the sporophyte, or non-sexual plant, which continues to increase in importance. Starting as a minute globular capsule completely filled with spores, there is first a sterilization of a part of the tissue which becomes devoted to the vegetative needs of the sporophyte. There is soon developed a special absorbent organ, the foot, and later a system of green tissue for the assimilation of CO_2. The sporophyte at last, in the ferns, by the development of a root, becomes entirely self-supporting, and from this time the sporophyte becomes the important phase in the plant's existence, the gametophyte becoming more and more reduced. The complete independence of the sporophyte is associated with the development of special organs, — and a corresponding perfecting of the tissues.

The sporogenous tissue, which at first constitutes practically the whole of the sporophyte, finally becomes restricted to special organs, sporangia, which are met with in all the higher plants. The development of the spores themselves, however, is extraordinarily uniform throughout, and is one of the strongest arguments for the common origin of all these forms.

The remarkable development of the sporophyte in the higher plants has been influenced by many factors, but probably the most potent was the abandonment of the originally aquatic habit characteristic of the primitive plants. Having assumed the terrestrial environment, many other causes combined to produce the marvelous structures which we see associated with the sporophyte of these higher plants.

SECOND LECTURE.

THE NATURE OF THE EVIDENCE EXHIBITED BY FOSSIL PLANTS, AND ITS BEARING UPON OUR KNOWLEDGE OF THE HISTORY OF PLANT LIFE.

D. P. PENHALLOW.

In directing attention to some of the more recent results in the study of those strange forms of plant life which flourished in past ages, and which are now known to us only through their fossilized and very fragmentary remains, it is my purpose to discuss some of the fundamental aspects of the question and endeavor to indicate the direction along which palæobotanists are working in their efforts to discover those facts which at present constitute the missing links in a phylogenetic chain, as we know it through a study of existing species; and in so doing I shall present a few typical cases in illustration of the nature of the evidence it is possible to secure from organisms long since extinct, and the remains of which have not only been broken up and the parts widely scattered, but which, during their entombment in the crust of the earth for enormous periods of time, have been subjected to the destructive influences of decay, usually supplemented by the infiltration of mineral matter, and those multiform alterations which follow from heat and pressure, consequent upon the great and varied changes in the material surrounding them.

Until recently the study of fossil plants has possessed few attractions for the rising botanist of the new school, very probably because he saw little opportunity for the development of the philosophical faculty and no great probability of discoveries of a higher order than the possible identification of a new species. The progress of botanical science in other directions, however, notably in our knowledge of anatomy and a more critical appreciation of the significance which attaches to the development of tissues and the growth of organs, has brought about a radical change in the point of view from which we to-day regard fossil plants. The very fact that we now apply to such remains, so far as their nature admits, the same exact and searching methods of study which are applied to living forms, and that in them we recognize the possibility of discovering the key to some of the most important problems in development which confront us at the present time, imparts to this branch of botanical work the same zest that is so marked a feature in other departments of the science, while the many and often great difficulties which surround the elucidation of any given problem seem but to strengthen the energy and keenness with which the pursuit is maintained. It is, therefore, a field which offers abundant attractions to an enthusiastic and diligent student; but for successful results it demands a broad foundation in the science as a whole, and especially an intimate knowledge of anatomy, both gross and minute.

The difficulties which surround the study of fossil plants are so unlike those to be met with in a study of existing species, that a brief consideration of their nature and bearings may serve to give a clearer conception of some of the facts which I shall later bring forward. These difficulties may be grouped under two principal heads:

1. The nature and position of the material.
2. The character of the alterations it has undergone.

1. *The Nature and Position of the Material.*

It seldom happens that fossil plants are found *in situ*. It is quite true that, in the overlying slates of coal beds, ferns and other characteristic plants are commonly met with, and are thus to be observed in the places occupied during growth. We also have similar examples in the erect Sigillarias of the South Joggins, Nova Scotia, or the more recent submerged forests to be met with at Leasowe and other places about the coast of Great Britain—examples of plants which have been preserved in place, and from which, therefore, we may draw important inferences respecting their former environment and habits of growth, but such examples are comparatively rare. The great bulk of the material with which the palæobotanist has to deal has been displaced, chiefly through the action of water, as exemplified at the present day by such great rivers as the Amazon, Mississippi, or Nile. The evidence of such transport is generally conspicuous and admits of little if any doubt. Thus, in the woods so abundant in certain Pleistocene deposits, the usually small fragments show in their worn surfaces and rounded angles all the characteristic features which can come only through long immersion in water and the prolonged action of waves, or transport over considerable distances. In the banks of the Moose and Missinaibie rivers, which run from near the north of Lake Superior to James Bay, extensive deposits of moss, lying under a depth of fifty feet of soil, and consisting chiefly of Hypnum and Distichium, occur. These deposits are characterized by the compressed, flaky form of the material,—the flakes being separated by silt consisting of sand,—and commonly enclosing fragments of wood which have been worn by the prolonged action of water. The evidence here indicates that the material must have had its origin near the head waters of the rivers, that it was carried down to a point near the mouth, and there deposited in pockets, as now found.

As, at the present day, vast quantities of plant remains are washed down the tributary rivers into lakes or into the ocean itself, so must we consider that the same process has been operative during all the ages through which the earth's crust has been undergoing continual change. Few examples offer more instructive lessons relative to the general conditions under which plant remains become buried by successive layers of sand and mud, and eventually converted into fossilized forms, than those which occur upon almost any ocean beach. Here we note in one enormous mass the commingling of land and marine plants which, in conformity to conditions of topography, wind, and currents, are commonly localized in a particular place, and there, through the repeated and long-continued action of the waves, are beaten into fragments mingled in the most inextricable confusion, until eventually covered by a deposit of sand or clay. Through all the long period of this process, decay has wrought destruction in the more perishable material, until that which finally passes into the fossil state represents a mere fragment only of the original material. The significance of such evidence becomes apparent when it is desirable to employ such remains as a test of climate, since the conclusions drawn must obviously depend upon whether the material is *in situ* or not, and if not, its original source.

Fossil plants are very rarely, if at all, found in anything like a complete condition. This naturally results from the destructive action of the water which serves to transport them, as well as from the unequal effects of decay and the operation of pressure and heat to which they are so often subjected. From this it follows that the mere association of parts, when organic union is wanting, is altogether untrustworthy as evidence of common origin or specific identity; and it most commonly happens that the restoration of a species can be accomplished, if at all, only after an exhaustive study of numerous fragments from different sources. Few such restorations are satisfactory; most of them are purely hypothetical with respect to many of their most important features, and they thus serve no final purpose; they are merely the expression of what we commonly term "working hypotheses." Large numbers of plants are known by their leaves only. This is particularly true of the Tertiary, as also of the Cretaceous flora, where, however, the fruit and sometimes the silicified or calcified stems also aid in identification. In the Devonian and Silurian they may be known by leaves, but more commonly by fragments of branching stems, by fruits, or by exceptionally well preserved stem structure. In recent formations like the Pleistocene, the stem structure may be so perfectly preserved as to admit of direct and exact comparison with existing species, and thus the genus, and often the species itself, may be determined beyond all reasonable doubt. It will be observed, however, that in the preservation of those parts, upon which the determination of relationship so largely depends, *viz.*, the more delicate structures of the gametophyte, we encounter one of the most serious difficulties in the prosecution of such studies. In the case of delicate plants or the delicate parts of woody plants, complete disappearance of structure commonly follows, and if any evidence of organic structure remains it generally appears in the form of highly altered carbon, as coal or graphite, or else, as usual in the case of leaves, only a mere impression of the external features is preserved in the surrounding material. It is altogether exceptional that, as in Parka decipiens, we meet with structures of so delicate an organization as the prothalli, and when such evidence is obtainable it acquires an unusual value.

2. *Character of the Alterations.*

Plant remains are rarely found in their original condition except in the most recent deposits, such as the Pleistocene. A very superficial examination of the floor of any well-grown forest, or of a forming peat bog, will serve to disclose the general conditions under which plants pass into the fossil state. Here we note that as the various forms of vegetation attain maturity and fall to the ground, they at once pass into a state of decay. If it then becomes possible for this decaying material to be covered by a fresh deposit of silt, secondary effects follow; the decay is either arrested or its character is altered, and very possibly the structure becomes infiltrated slowly with mineral matter which is held in solution by the surrounding water. That such conditions have surrounded the fossil plant is evident from the form in which it is found.

Decay, more or less pronounced, is an invariable antecedent of the final preservation of plant remains. In many cases it has been carried to an extremity, so that the structure is unrecognizable, and then there remains nothing but a residue of carbon, as so commonly occurs in the Eozoic formation. In other cases it may be so limited as to permit a recognition of all the original structural features preserved with great perfection. The fungi of decay are commonly met with, and are not only represented by their mycelia, but, as shown by Renault and others, even the bacteria are now regarded as well-known forms among carboniferous plants.

Plants which are enclosed in a deposit of compact clay while yet living, or before any essential decay has taken place, often exhibit the most perfect preservation, since they have been hermetically sealed, and thus have retained all their structural features in an unaltered condition for an unknown number of thousands of years. Notable examples of this occur in the various interglacial deposits. In the Leda clays at Montreal, when fresh cuttings are being made, one may see leaves of the common Vallisneria spiralis as perfect in all their structural features as if freshly gathered from the adjacent river. But the delicate structure has been so far influenced by its long enclosure in the moist clay that desiccation leaves nothing but a mere impression of the former organ. In the same formation near Ottawa, fragments of perfectly preserved wood are enclosed in nodules of clay, while at Toronto, from the same formation again, we obtain the Osage orange with all its structure perfectly preserved, and red cedar, which is not only perfect as to structure, but which possesses the characteristic shreddy bark, red color, and distinctive odor. Such woods are sectioned in the microtome precisely as if taken from a living plant.

While woods from the Pleistocene are often preserved as just described, it more commonly happens in older formations that the mass has been wholly or in part carbonized, or that it has become so charged with mineral matter that it has passed into a petrified state. These two forms of alteration commonly accompany one another.

(a) *Carbonization.*— Plant remains which are covered shortly after decay arises, so that the latter continues under exclusion of air, become converted into a mass of carbon which, according to circumstances, retains the original structural features more or less perfectly. This carbonization results from withdrawal of the elements of water under the peculiar conditions established, and if pressure and heat are subsequently brought into operation, there results a compact coal, and possibly certain oily and gaseous products, as may be noted in the case of the Ohio shales, which are remarkable in some localities for the vast number of spores of Protosalvinia, associated with oil, and in such a state of preservation that they may be recognized very readily. The extreme form of such alteration is to be found in graphite, while the various forms of anthracite, bituminous coal, lignite and peat indicate in inverse order the successive stages through which the plant remains pass. It follows from these considerations that, as a rule, we can expect little structure to be exhibited in coaly masses, but we may draw correct conclusions as to the general character of the parts represented by taking into consideration the position in the specimen and the original nature of the various tissues. Thus we know that unmodified cellulose, such as constitutes the soft parts of plants generally, contains carbon 44.55%, hydrogen 6.14%, and oxygen 49.51%. Lignin, or the essential basis of all woody structures, contains C. 62.25%, H. 5.93%, O. 36.82%, while cutin and suberin, the basis of cuticle, the walls of spores and of cork, contain C. 73–74%, H. 10%, O. 16–17%. A careful consideration of these figures leads to most important and interesting conclusions, since the durability of parts, or their ability to resist decay, as also the readiness with which they pass into the form of carbonized remains, is in direct proportion to the relative excess of carbon in the original structure. Hence it is not difficult to understand why the more perishable fundamental tissues of plants are so rarely preserved while the woody and cortical parts are well preserved, and why the latter particularly may remain as a shell of coal when all other parts have been removed by decay or replaced by mineral matter. In the light of these facts, also, the remarkable preservation of many spores may be readily understood.

As carbonization proceeds, the material may become saturated with water holding in solution small quantities of carbonate of lime or of silica, in which case the entire structure becomes gradually converted into a mass of calcite or of silica, as the case may be, the displacement of the organic matter proceeding at so gradual a rate that all the structural features are retained. The structure is then represented by fine particles of carbon disposed along the original lines of structure, while the various cavities are filled with the calcite, silica, or other infiltrated material.[1]

Plants from the early Palæozoic are often most beautifully preserved in this way, but cases occur in which the recrystallization of the infiltrated material brings about a redistribution of the carbon particles in such a way as to produce a false structure, as found most notably in the Celluloxylon primævum of Dawson, which was originally supposed to represent a purely cellular plant, the component cells of which were of gigantic size. More recent studies of this material, and comparison with other well-determined plants in various conditions of alteration and petrifaction, have afforded ample proof that the apparent structure of Celluloxylon is nothing more nor less than a condition incident to petrifaction, in which the carbon of the original structure has been redistributed through the influence of crystallization and deposited upon the surfaces of, or otherwise between, the adjacent crystals.

Two full centuries have passed since the first observations upon the occurrence of fossil plants were made. Yet palæobotany has only recently attained to a position commensurate with its importance as a branch of botanical science from which most important and necessary data respecting the gradual evolution of the higher plants may be gained. During the seventeenth century, when men were still experiencing the influence of the Middle Ages, the discovery of plant and animal remains in the crust of the earth was well calculated to call forth in all seriousness the most remarkable explanations of their occurrence. And among other things we are thus told that such evidence gave proof "that the whole terrestrial globe was taken all to pieces and dissolved at the Deluge, the particles

[1] A most instructive example of the extent to which a given material may be involved in the process of petrifaction is afforded by Osmundites skidegatensis Penh. from the Cretaceous of Vancouver Island. An analysis shows it to contain

Calcite	70.46%
Silica	12.15%
Combustible matter	17.36%
	100.00%

The last constituent which disappears upon ignition, leaving a calcined mass, probably represents the amount of carbon residue derived from the original plant.

of stone, marble, and all solid fossils discovered taken up into the water and there sustained together with seashells and other animal and vegetable bodies ; and that the present earth consists and was formed out of sand, earth, shells, and the rest falling down again and subsiding from the water."

We are also informed that "The Deluge came forth at the end of May when nuts are not ripe," because of the occurrence of imperfectly formed hazel nuts in certain moss beds. Even half a century later the idea that such remains were proof of a deluge had in no way lost its force, since da Costa, who first pointed out that Sigillarias and Stigmarias represented unknown forms of life, and was, therefore, the first to indicate the extinction of former types, firmly believed cones to be of vegetable origin buried in the strata of the earth at the time of the universal deluge recorded by Moses.

In these views we, no doubt, have an expression of the survival of primitive beliefs which are still current among certain aboriginal people of eastern Asia.

And so for fully a century and a quarter the remains of plants buried in the crust of the earth for millions of years were matters of speculation without any adequate conception of their real significance. Since the time of Witham, Sprengel, and Goeppert there has been a constantly increasing interest in the scientific study of plant remains and a correspondingly greater appreciation of their true bearing upon the history of plant life. Of the pioneers in this work, among English-speaking people, probably no one has done more than Williamson in England and Sir William Dawson in Canada to emphasize the primary importance of the internal structure as a true guide to relationship.

Our present knowledge of living forms leads us to the conclusion that there has been a more or less regular succession of types from the most simple to the most complex ; and this has been brought about, not by acts of special creation, but by the gradual evolution or unfolding of continually higher types in direct response to changed conditions of environment. What those conditions were we are unable to say, but from our present knowledge of plants in respect to their environment we are permitted to draw certain inferences as to the general nature of the controlling forces. That such changes may, in particular cases, be brought about quickly, so that the results become more or less apparent within the space of an average life, and thus form the basis of broader generalizations, is well known; but in the main they have progressed but slowly, and enormous intervals of time have been required for the transition from one type to another. We are not permitted to conceive that this line of descent takes the form of a strictly lineal succession. On the other hand, there is strong evidence in support of the view that our mental figure must be that of a deliquescent tree in which the main line of descent, or stem, is dissolved into a series of great limbs or primary divisions. These again branch repeatedly, until in the final division all direct connection with the original stem is lost. As certain of these lesser branches retain a full measure of vigor and persist in their development to the very end, so others attain their highest development at a comparatively early stage, enter upon a period of decline, and shortly disappear, thereby introducing a further important disturbance of the natural arrangement whereby it becomes increasingly difficult to determine the precise order of development and relationship between any two members. And so with plants as a whole. Certain side lines of descent have not yet attained their full development; others are now far along in their period of decline, which may have had its origin in very remote geological times; while yet others have long since disappeared altogether, leaving no visible sign of their former presence. And thus the symmetry of the biological tree is disturbed to such an extent and in such a way that the remaining members appear to have little in common, and sometimes even stand forth as isolated groups which seem to justify the ancient idea of special creation. Fortunately the situation is saved, in the first instance, by an appreciation of the fact that, within certain limits, each individual reproduces in the course of its life history the history of the group. From this it follows that, however divergent species or groups may appear in their fully matured state, the relationship may be ascertained through their embryological phases and through their most elementary members; and thus it becomes possible to ascertain at what points suppression has occurred and the normal succession been broken.

We are perhaps not far wrong in the assumption that the general line of descent began with the green algæ as the first clearly defined type. From these aquatic forms, which were, no doubt, at first dominant, if not the exclusive forms, amphibious types appeared, leading eventually to terrestrial forms, as represented by the mosses and liverworts, plants which clearly show their derivation from an aquatic ancestry when they enter upon their reproductive phase, and especially in the development of an algoid protonema. But here we encounter one of the so-called missing links, since, although the approach of the alga to the moss, and of the moss to the alga, is well defined, the intermediate stages are as yet unknown. And so again the thallus of the liverwort reappears in the prothallus of the fern, and as these latter lead on to higher types, we find undoubted evidence of relationship, the exact bond of which is as yet wanting. One of the most significant facts, however, is to be found in the evidence of an aquatic ancestry, which extends through all grades of development in terrestrial plants until the Cycads are reached; I refer to the occurrence of motile spermatozoids, the significance of which can scarcely be misunderstood.

It is highly probable that further light respecting these obscure problems will be gained as our knowledge of living forms advances; but as many of the existing gaps in the biological tree have resulted from suppressions which must have occurred in remote periods of the earth's history, it is peculiarly within the province of palæobotany to discover the necessary data, and thus supply the missing links in the chain of plant life, in order that we may have a clear and complete explanation of the relations of the various great groups of plants. And it is among those forms which are now extinct that our search must be prosecuted with the greatest hope of success. That this is within the limits of possibility has been abundantly shown by the investigations of recent years, and possibly no better illustration could be had than the evidence derived from extinct forms as presented by Dr. Jeffrey in his recent admirable memoir on "The Development, Structure, and Affinities of the Genus Equisetum."

Among the most interesting and promising of all botanical problems is the theory relative to the origin of the sporophyte as propounded by Bower a few years since, and more recently emphasized by Campbell, who has himself done so much to advance our knowledge in this direction. While the evidence to be derived from living plants points with great force to the possible correctness of Bower's hypothesis, it is not yet so complete as to justify us in regarding the law as fully established. But it is in the solution of problems of precisely this nature that palæobotany would prove of the highest importance, and it is probably not too much to expect that the study of fossil plants, particularly of types now extinct, may eventually enlarge our views upon this as upon other important problems.

If we now turn to plants as we find them in the rocks, it is to be observed that the succession displayed by living forms is there essentially repeated and thereby confirmed. A glance at the geological succession of the earth's crust shows that there are four great periods in which geological time may be reckoned, and that these periods conform in the main to epochs in the development of plant life. In the earliest or Eozoic time, chiefly represented in northeastern America by the great Laurentian formation which gives the dominant physical aspect to the northern watershed of the St. Lawrence River throughout the greater portion of its length, there are few and trustworthy evidences of former plant life to be obtained; that is to say, we find in those rocks no well-defined plant remains. On the other hand, the occurrence in the Palæozoic of plants of a somewhat high degree of organization leads to the inference that they represent a line of descent which must have had its origin very early in Eozoic time. But if definite remains are wanting, there is nevertheless evidence in the abundance of graphite which occurs in the Laurentian formation, commonly interstratified with gneiss and attaining a vertical depth upwards of six hundred feet or more, of former vegetation; for, as Prestwich very correctly observes, there is good reason for the belief that the various forms of carbon now found in the crust of the earth must have been derived in the first instance from the atmosphere through the agency of green plants. Sir William Dawson has also expressed the opinion that if the Laurentian graphite may be taken as representing former plant life, it indicates its occurrence in vast profusion, and this was no doubt the case. To these views we may also add the opinion of the late Sterry Hunt, that the great Laurentian beds of iron ore had their origin in plant decay, precisely as they are formed at the present day.

During those early times the water resting upon the earth's surface was of a rather high temperature. At the same time the atmosphere was charged with a relatively high percentage of carbon dioxide, in consequence of which its temperature was considerably higher than at present. These considerations lead to the conclusion that the earlier and aquatic vegetation — of the general character of the Chlorophyceæ with which we are familiar to-day, but whose delicate structure did not admit of permanent preservation — flourished under conditions now fairly represented by hot springs, where vegetation thrives at temperatures upwards of 93° C. If, furthermore, plants began to emerge from their aquatic condition in later Eozoic time, they must have been brought under an environment essentially similar to that of the tropics of to-day. Certainly this was the case until late in Palæozoic time.

We are thus led to the belief that the flora of Eozoic time was not only very abundant, but that it consisted, perhaps wholly, of aquatic thallophytes comparable with the marine and fresh-water algæ of to-day ; that those plants were capable of photosynthesis, and that along certain lines they attained a somewhat high degree of development.

One cannot fail to remark upon the peculiar absence of plant life during Huronian and Cambrian times, while evidences of animal life are abundant. This may have its explanation in the complete destruction of the more perishable plant structures, consequent upon the great disturbances which characterized the close of the Eozoic and the opening of the Palæozoic period. That plant life must have been maintained in full vigor during that time is evident from the nature of the remains which appear in later formations, and the first well-defined occurrence of terrestrial forms in the Siluro-Cambrian or Ordovician would go far to show that plants passed through their amphibious stage at least as early as the Huronian, thus preceding the corresponding phase in the progress of animal life by a very long period of time, since in the latter case this change was not effected until the later Carboniferous and Permian. That the Eozoic and early Palæozoic must have witnessed the development of high types of plants is abundantly evident from the abrupt occurrence, in the Upper Silurian and Lower Devonian, of gigantic marine algæ, of which there are no equally large representatives in later formations; while in the Devonian, also, there occur for the first time plants which are comparable in their reproductive structures with the more modern Pilularia, and a brief consideration of the leading features of some of these plants may assist us in gaining a more complete appreciation of the statements already made.

In his *Old Red Sandstone*, Hugh Miller described certain peculiar discoid bodies found in the Devonian rocks of Scotland at Blairgowrie, Myreton, and other localities. In 1831 these objects were described by Dr. Fleming under the name of Parka decipiens, and both by him and subsequent observers, during a period of sixty years, they were regarded as representing the spawn of Mollusca, the eggs of frogs or other animals, but always without suspicion that they might have been derived from plants; and it was reserved for Dawson and Penhallow, in 1891, to prove clearly their vegetable nature. These bodies consist of carbonized discs, or their impressions, about 5–6 mm. in diameter and grouped in oval masses varying in size from 3.5 x 5.3 cm. to 13 x 20 mm. In their more complete forms these masses show the presence of an external covering, and there is also some indication that a stalk may have been present. No stems or leaves can with certainty be considered to belong to these bodies, since as yet no organic union between such organs has been observed, but there is reason to believe, from the associated structures, that they belonged to plants with a creeping stem and upright leaves, while the recent finding of leaf-like bodies similar to those of Marsilia seems to suggest the possibility of foliage of that type. Our present knowledge, however, centers in the carbonized discs, from which important data have been obtained. Boiling them out in nitric acid they yield two kinds of spores. The macrospores measure 40 μ in diameter and are therefore slightly larger (34 μ) than the spores of Lycopodium. The somewhat elongated microspores are 15 μ in diameter. In addition, there are to be found numerous compound cellular bodies with a central carbonized mass. The obvious remains of spores, from which as a center there extends a tissue in various stages of development, leave no doubt as to the fact that these structures represent prothalli in various stages of growth. From these facts the conclusion is a direct and justifiable one that Parka was a heterosporous plant with its separate male and female sporangia enclosed in a common sporocarp, and therefore comparable among modern plants with Pilularia. Beyond this nothing definite can be stated, hence for the present the specific name decipiens, as originally applied by Fleming, is retained.

In 1855 Sir William Dawson discovered, in the Devonian sandstones of Gaspé, the remains of a gigantic alga, to which he subsequently gave the generic name of Nematophyton. Since then plants of the same type have been found in Germany, England, Scotland, and various parts of the United States, thus indicating the very wide distribution of plants having a common ancestry. At the present time, for purposes of convenience, eight different species are distinguished, but it is altogether probable that these may in reality represent only four separate species at the most.

As represented by specimens of N. Logani in the Peter Redpath Museum of McGill College, these plants were sometimes eighteen inches to two feet or more in diameter, and specimens recently found in New York State show a recoverable length of twenty-four feet. These facts seem to indicate that the complete plant attained to great size, far exceeding anything known among the arborescent forms of algæ at the present day. No foliage or fruit has been found, and our knowledge of these plants rests entirely upon the details of internal structures, which are often most beautifully preserved. The main features are as follows:

The stem exhibits a concentric structure comparable with that which is shown by the larger Laminariæ, a feature which in the first instance led to a misinterpretation of their true nature, as it appeared to indicate an exogenous stem. This view was still further strengthened by the appearance of certain radial canals simulating medullary rays, but of very irregular width and indeterminate length, and wholly lacking definite, limiting walls. Such spaces reappear in N. Ortoni, N. Crassum, and others, where they take the form of nearly isodiametric openings in the body of the structure, through which they are scattered without any definite order or apparent connection with the larger elements of the organism. They nevertheless appear to be intimately connected with the smaller and secondary elements, as will be shown presently, and it is therefore quite possible that, as suggested by Barber, they were designed to perform a certain rôle in the internal aëration of the plant.

The principal structure consists of tubular, non-septate cells having a diameter upwards of 67 μ, but of indeterminate length and loosely interlaced, so that their general direction coincides with the axis of growth. These large cells of the medulla branch into smaller hyphæ which form an intercellular plexus. The hyphæ are chiefly from 4–6 μ in diameter, though sometimes much less. They appear, in some cases at least (N. Storriei), to be septate, and their derivation from the large cells of the medulla appears to occur chiefly in the immediate region of the medullary spaces which they occupy in the form of a very loose web. The majority of the species exhibit minor differences only, but in N. Ortoni we observe the occurrence of well-defined trumpet hyphæ, which not only establish a certain degree of affinity with algæ of the type of Macrocystis, but also serve to suggest its possible generic separation from the other known forms.

Our interest centers in the fact that these plants have no modern representatives in any way comparable with them in point of size. Occurring as they did so early at the Upper Silurian, their gigantic dimensions and somewhat highly specialized structure suggest that they represent a line of descent which must have had its origin very early in Eozoic time.

The limits of time and space will not permit of our considering the many problems suggested by our title more at length, but, in conclusion, it may be well to point out that as, with the progress of the ages, plants became less and less aquatic in their habits, they were brought more and more completely under the influence of conditions which became continually less stable, in response to the lower temperature and changing surface of the earth's crust. This eventually led to waves of vegetation which passed over the great land areas, from the direction of the poles toward the equator and back again, in direct response to changing climatic conditions. Such migrations are well known as having occurred during glacial times, and the evidence of this fact, as presented by fossil plants, especially those of the Pleistocene age, has an important bearing upon our knowledge of the distribution of plant life, as well as upon the general history of development.

THIRD LECTURE.

INFLUENCE OF INVERSIONS OF TEMPERATURE, ASCENDING AND DESCENDING CURRENTS OF AIR, UPON DISTRIBUTION.[1]

PROF. D. T. MACDOUGAL.

THE soil and the air receive the same amount of heat from the sun during the course of the day, but the former, on account of its greater conductivity, becomes warmer than the air resting upon it. Both the soil and the air begin to lose heat shortly before sunset, but the former undergoes cooling much more rapidly than the latter, for the same reason that it becomes warm more rapidly. If the air is poor in moisture, showing a low relative humidity, it will conduct heat very slowly. As a consequence of this fact, the layer of air, a few meters in thickness, nearest the ground will become cooled by conduction and radiation to the cold surface of the soil, and soon falls to a temperature many degrees below that of the air a few yards above. This is termed inversion of temperature by the meteorologist, and the effects of inversions of temperatures are well known to those engaged in horticultural and agricultural operations.

The late spring frosts and the earlier frosts of the autumn are generally due to such inversions of temperature. It is a matter of common observation in these occurrences that the lower branches of trees and shrubs will be killed, while the topmost buds will be unharmed ; showing that the layer of cold air was not deep enough to submerge the entire plant. As a

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[1] Delivered August, 1899. Based upon investigations reported to the U. S. Department of Agriculture in 1898. Printed by permission from the chief botanist.

recent example of such action, a description of the manner in which plants were injured by a late frost in Florida illustrates the matter quite fully (*Botanical Gazette 18*, p. 417, 1893). In this paper it is noted that all of the young shoots on the limbs of the china tree, *Melia Azedarach* L., "less than eight feet" from the ground, were destroyed, while those "above twelve feet" were left unharmed. The young shoots on the lower part of shrubs of the prickly ash, *Xanthoxylum clava-herculis* L., were destroyed, while those "above twenty feet" from the ground were uninjured. All the young leaves of the mulberry, *Morus alba*, within ten feet of the ground were killed. These figures show us the depth of the layer of cold air, with a temperature below the freezing point, as plainly as if we had tested it with a recording thermometer. Very naturally the effects of inversions of temperature are felt only on clear, still nights. A wind would prevent the accumulation of the layer of cold air, and a shield of low-lying clouds would act as a reflector in preventing the escape of the heat from the ground at the usual rate. The device of the orchardist and farmer in building smoky fires at intervals in his crops would both give rise to local currents, which would prevent the accumulation of a cold layer, and would also simulate the presence of clouds; constituting, in fact, a very effective device for the avoidance of frosts. Coverings, however slight, such as paper or of the thinnest and lightest cloth, would also prevent the greatest loss of heat from the soil underneath the plants thus protected.

These inversions of temperature occur practically over the entire temperate zone in a manner of importance to the plant, and this phenomenon accounts for the low night temperatures prevalent on all the elevated plains in western North America, in which the effect is emphasized by the comparatively low relative humidity.

The frosts resulting from this action are not to be confused with the freezing which ensues when great waves of cold air sweep over a section of the country.

In the summer of 1898 I carried out some ecological observations for the United States Department of Agriculture on the plateau, inclusive of northern Arizona, and lying at an elevation of about 2300 meters. I found the nocturnal inversions of temperature very marked and more or less constant — a fact which must exercise a very notable influence upon the zonal boundaries which cross this region. As will be shown presently, northern plants would go farther south on a plain subject to inversions of temperature than one free from such influences.

Quite early in the course of my investigations attention was drawn to the fact that crops of oats, wheat, and vegetables in the valleys around the San Francisco Mountains were frosted, while those on uplands and ridges were unharmed. In order to determine the temperature relations thus indicated, recording thermometers were placed in the valley at Flagstaff and at the Lowell Astronomical Observatory, 100 meters above it, and continuous records of the temperatures were made through June and a part of July. The record of a few days is shown in figure 1. From the data thus obtained it is to be seen that the valleys and cañons are subject to much greater extremes of temperature than the adjoining hills and mesas, and that the average daily temperature of the valley is lower than that of minor elevations near it. The temperatures of the hill and

September 19–22.

July 18–23.

FIG. 1. — Temperature curves obtained at Flagstaff, Arizona, at elevations of about 2300 meters. The dotted line is the thermographic record for the hilltop, and the unbroken line is the thermographic record for the valley, 100 meters lower. The valley is warmer than the hilltop during a period of only about three or four hours during the middle of the day, but its temperature is lower than that of the hill during almost the entire night of twelve hours. The daily average temperature of the valley is thus much lower than that of the hill.

valley begin to fall shortly before sunset, but continue with such unequal rapidity that the difference is very great immediately before sunrise. Thus, for instance, on the morning of Sept. 18, 1898, the temperature of the hilltop was 62° F. at 6.45, while that of the valley was 35° F., showing a difference of 27° F. between two points which differed only 100 meters in elevation, and which were actually so near each other that one could walk from one instrument to the other in ten minutes.

If the curves of the thermograph are examined, it will be found that the valley becomes from 3 to 8° warmer than the hilltops during the middle of the day.

These differences of temperature of the valleys and highlands are due to the action of currents of air set up by the inversions of temperature and by the direct action of the sun's rays.

If the surface of a region were perfectly level, the surface layer of air cooled by inversion would remain in place, and a fairly uniform temperature would prevail over the entire area. The cooling of the lower layer of air results in its contraction and consequent increase in weight, with the result that in regions with broken or irregular topography the cold air on the mesas, ridges, and hilltops flows down the slopes into the cañons, valleys, or other depressions, forming a deep layer, while a constant supply of warmer air settles down on the highlands, with the result that these minor elevations have a higher average temperature and a more equable climate than the valleys below.

The influence of this drainage of cold air has long been recognized by the farmer and horticulturist in the northern and New England states. Thus it is customary to select ridges and uplands for the growth of vineyards, orchards, and small fruit plantations in broken countries, since these places are less subject to frost than the lowlands.

If the air cooled by inversion on the highlands descends by long, continuous, steep slopes and drains into narrow valleys, it may exercise exactly the reverse influence upon the temperature of the valley. As it descends it will increase in temperature at the normal adiabatic rate, by compression, and actually reach the valley or plain as a warm wind, if it has had a descent of a few thousand feet. As a fall of sixty meters gives rise to an increase of temperature of 1° F., the total amount may be distinctly noticeable, although but few actual observations of this phenomenon are recorded. If the cooling slopes descend gradually to the plain, the heat lost by radiation in the slow downward progress of the current may entirely compensate for the adiabatic increase, and the breeze will reach the lowlands with no marked change in temperature.

A probable example of this adiabatic increase of temperature of a descending current of air is to be seen in some of the thermographic records made at Flagstaff, Arizona, by myself. This place lies in the mouth of a great valley, enclosed on one side by a mesa, on the other by a spur of the mountain, and on the other the valley gradually ascends the long slopes of the San Francisco Mountain, reaching the summit of Humphrey's Peak, twelve kilometers distant, at a height of over 4000 meters. As previously described, the cold air from the near-by mesa and ridges pours into the valley, steadily reducing its temperature through the night. The downward currents also form on the long slopes of the mountain, but, descending very slowly, do not show any great rise in temperature, and usually do not occasion any marked disturbance in the cooling of the valley. At certain times, however, the descending current gains such strength and rapidity that its increase in temperature overbalances radiation, and it reaches the valley in the latter part of the night as a warm current which causes an upward bend of the thermographic curve, which is steadily falling. The rise due to this warm current may amount to 2 to 5° F. at some time between 2 and 5 A.M.

The heating of the layers of air in contact with the soil in valleys and cañons during the daytime generally results in ascending currents, which, cooling at the normal adiabatic rate as they ascend, blow over the adjacent mesas and hilltops as cool winds. This reduces the maximum of the highlands, and in the curves taken on the Observatory hill at Flagstaff it is seen to be 2 to 6° lower than that of the valley below. It is thus to be seen that the nocturnal inversions of temperature and the diurnal ascending currents complement each other in reducing the extreme of the temperature of the highlands and in increasing the daily variation of the cañons and valleys.

The phenomena discussed in the preceding paragraphs are well known to the meteorologist, and may be found more or less thoroughly exploited in text-books upon that subject, and the above lengthy consideration of them is given in order that their application to the distribution of life and the limits of zones may be better and more easily comprehended.

It is presumably a fairly established conclusion with biogeographers that the northward distribution of southern plants and animals is governed by the sum of the positive temperatures for the entire season of growth and reproduction, checked of course by the low temperatures of the winter season. Positive temperatures are computed by adding the excess of temperature above the point at which plants start into activity in the spring. The season during which the positive temperature would be of moment would embrace only a few weeks of the hottest part of the summer (Merriam, "Life Zones and Crop Zones," *Bull. No. 16*, U. S. Dept. of Agriculture, Div. of Biol. Survey). The limiting low temperatures might occur at any time in the winter season.

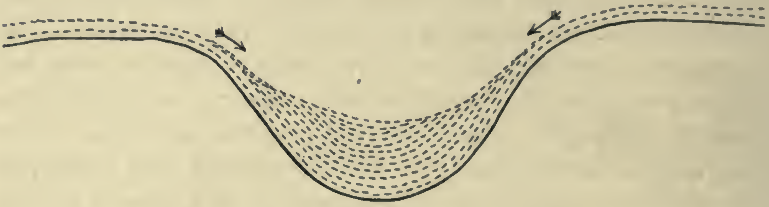


FIG. 2. — Showing drainage of cold air into a valley.

It is also generally accepted that the southward distribution of northern plants and animals is governed by the mean temperature of a brief period during the hottest part of the year.

Now it needs but a glance to bring us at once to the conclusion that the valleys have a lower mean temperature and a lower minimum temperature than the adjoining hills, and that they are therefore better suited to the southern advance of northern species than the reverse. Again, the higher minimum temperatures of the ridges and hills, and the greater sum of their positive temperatures would eminently fit them for the northern advance of the southern species. Given then a great plain, cut by valleys and cañons, or the foothill region of a mountain range, it may be definitely asserted that the southern plants reach their northern limit on the highlands, and the northern species reach their southern limit in the lowlands. Stated in another form, it may be said that zonal boundaries are deflected southward in valleys and northward on ridges and highlands. This conclusion applies, of course, to topographical features only which show differences in elevation of not more than 300 to 500 meters. Even with this limitation the configuration of the country may be such as to deflect the zonal boundary from its general course 100 kilometers or more; a fact of very great importance both in biogeography and also in economic operations.

The conclusions reached in the last paragraph are amply supported by the observations of the writer in the San Francisco Mountain region in northern Arizona, also by numerous facts of the distribution of plants and animals in an adjoining region in New Mexico, which have been kindly cited me by Professor Tyler-Townsend.

Large numbers of species of northern plants are to be found in the valleys of the San Francisco Mountains, while many southern forms are to be collected beyond the general outline of their zone on the southern mesas, hills, and spurs. The most marked illustration of the descent of the northern flora is to be found in the crater of the San Francisco volcano. The eastern wall of the crater has been broken away at some time in the last stages of its activity in Tertiary times, and the crater is now a U-shaped valley, ten kilometers long and three to five kilometers wide, heading up on the inner slope of the mountain at an elevation of 3500 meters, and opening out to the eastward on the plain at an elevation of about 2500 meters. According to current conclusions the species of the pine or transition zone should advance up this valley, especially along the face of the northern slope or side of the valley which has the most favorable slope exposure. The effect of the slope exposure should be such as to advance the transition flora about 300 meters higher along the northern wall than along the southern. As a matter of fact, the effect of slope exposure is totally lost, and the boundary of the transition zone is pushed backward and downward 300 meters below its normal level.

Professor Tyler-Townsend states that similar illustration of this principle is to be seen in the Mesilla valley, a portion of the valley of the Rio Grande in New Mexico. The valley has an elevation of 1200 meters and rises gradually on the east to an elevation of 1700 meters, on the slopes of the Organ Mountains fifteen kilometers distant. As a consequence of the meteorological phenomena detailed above, *Larrea tridentata* advances northward along the mesa above elevations of 1300 meters, while it cannot grow in the valley 100 meters below. At the same locality he reports the presence of a peculiar fly, *Raphiomidas xanthos* Towns., of the lower Sonoran fauna, not found elsewhere north of Lower California. *Volucella opalescens* Towns., a member of the subtropical group of the genus, and *Lecanium imbricatum*, a distinctly neotropical type, also occur in this locality. *Volucella haagii* Jacun. occurs on the mesa in question, as well as in similar locations in the White and Magdalena mountains, where it is beyond the general curve of its zonal limits.

That this distribution is entirely the result of the meteorological factors, chiefly inversions of temperature and cold-air drainage, seems to be indicated by the fact that genera of flies such as *Gymnosoma*, *Hyalomyia*, *Trichopoda*, *Cistogaster*, *Hemyda*, and *Ocyptera*, which range from the transition zone to the tropics, are found both on the mesa, along with the other subtropical forms, and also in the valley, showing that food supply or other conditions of environment do not account for the northward advance of the southern forms enumerated.

The facts which have been brought before the writer in the investigations described above lead to the belief that many of the so-called aberrations of distribution which have been recorded in various parts of the world may be found directly attributable to the influence of the ascending air currents, although it is well known that species of plants and animals often form isolated colonies outside the natural boundaries of their zones because of special or highly local conditions of dissemination agencies or food supply.

Incidentally, it is believed that these meteorological factors may have some connection with the variations in the depth and tint of the autumnal colorings of foliage leaves, though the apparent evidence may be entirely coincidence.

Aside from these phenomena which deflect the zonal boundaries of plants and animals, I now wish to call your attention to certain movements of the air which may be said to have a purely local selective effect.

Among these are the chinook or foehn winds, which are prevalent in certain localities over the world, being especially well developed in western North America, and the moist ascending currents which exercise their greatest influence in regions with low relative humidity.

Chinooks are formed to the leeward of nearly all high mountain ranges, and are heaviest near those which stretch their barriers directly across the course of the prevailing winds. The factors necessary to produce a chinook consist chiefly in the difference in barometric pressure on the opposite sides of the mountain ridge, a high-pressure center to windward and a low-pressure center to leeward. The ascending current of air which flows over the crest to restore the equilibrium loses much of its moisture in the elevating, expanding, and cooling processes, and then as it descends the opposite slope, undergoing compression, its temperature increases at the normal adiabatic rate of 1.6° F. for every 100 meters of descent, and reaches the lower slopes as a warm, dry wind, comparatively speaking. The temperature of such winds is rarely above 50° F., but they occur at a time when the general temperature is near or below the freezing point.

Under the influence of this high temperature many plants are induced to start into activity, and as these chinooks last only a few hours, the restoration of the general average temperature results in the destruction of the partially opened buds and active growing plants, while the direct desiccating action of the warm wind itself is often very injurious. The presence of the chinook results in the elimination of the species which are particularly susceptible to the warm, dry air from the localities affected.

A few years since, the leaves of coniferous trees on the lower slopes of Pike's Peak were killed in this way, and the San Francisco Mountains offer marked examples of this action. On the eastern slopes of the principal elevation of this group the coniferous forest reaches an elevation of 3000 to 3500 meters. On these slopes, in the localities which are exactly suited to receive the strongest action of the chinook, are extensive areas of pine and spruce trees which, so far as repeated examination by myself and others shows, were not destroyed by fire or disease, and the supposition is fairly allowable that the chinook was the destructive factor. This supposition is strengthened by the fact that the small hills and volcanic cones which stand directly to the eastward in the path of the winds lose their snow before other places of the same altitude and geological formation elsewhere in the region.

In addition to the action of the ascending currents of air on the temperature of highlands they often occasion serious disturbances of the moisture conditions in a highly localized manner.

The ascension of a current of warm air from the bottom of a valley or cañon necessitates the loss of some of its heat to furnish the energy for expansion, and the consequent cooling entails the lowering of the dew point. This action may proceed until the point of saturation is reached and precipitation occurs. More generally, however, the current reaches the highlands as an extremely moist wind. The margins of mesas and hilltops adjoining deep valleys are most subject to such moist winds, and as a consequence these localities offer suitable conditions for moisture-loving species which thus may occur in great abundance as oases in an arid region. As the moisture-laden current traverses the high plain, it soon becomes heated to the temperature of the air through which it is flowing, and its relative humidity is decreased.

The moisture influence of ascending currents is strikingly illustrated in the region of the Coconino forest bordering upon the famous Grand Cañon of the Colorado River in northern Arizona, where many moisture-loving species may be found fringing the rim of the cañon, which are not to be seen in such abundance elsewhere on the mesa. *Razoumofskya robusta vaginata* (Willd.) *Kuntze* is a loranthaceous parasite growing on the branches of the bull pine (*Pinus ponderosa var. scopulorum*) throughout the transition zone. It is most abundant, however, along the margins of mesas, the rims of cañons, and hilltops, and reaches its greatest density along the Grand Cañon on the Colorado River. Here the heated air, rising from the river under the influence of the subtropical sun, loses about 20° F. in its ascent of 2000 meters, and as a consequence it pours over the mesa much cooler and with its relative humidity increased to near the point of saturation. The *Razoumofskya* finds this strip of territory, where the effect of the moist air is greatest, most advantageous in the germination of its seeds and the attachment of the seedlings to the host plant. It is therefore most abundant in a belt one or two kilometers in width, running parallel to the rim of the cañon, while it is comparatively infrequent at greater distances. Within this belt it is estimated to have gained a foothold on more than half of the pine trees.

In conclusion, I must again remind you that none of the meteorological principles described are in any sense original with myself; but I am able to adduce some very striking observations in illustration of their influence upon vegetation, and it is confidently believed that this work constitutes the first systematic attempt to use such data in explanation of certain seeming aberrations of distribution and zonal boundaries. Furthermore, it is most interesting to note that the effects of cold-air drainage and inversions of temperature have been taken into consideration by the horticulturist and farmer long before this analysis of their relations to the facts of natural distribution of plants and animals was brought forward.

FOURTH LECTURE.

SIGNIFICANCE OF MYCORRHIZAS.

PROF. D. T. MACDOUGAL.

By reason of the great adaptability of the seed plants, any underground member, roots, stems, branches, or leaves may come to serve as organs of absorption of liquid nutriment from the soil in different species. Furthermore, because of this readiness to adopt any method which will enable them to acquire food more economically, the absorbing organs of an extremely large number of species have acquired the habit of forming symbiotic unions or partnerships with the hyphæ of soil fungi. Such unions are termed "mycorrhizas." But mycorrhizas are not confined to the absorbing organs of the higher plants. The roots of the sporophytic generation of a number of ferns and fern-like plants also form similar structures, while the wide occurrence of fungi in the thallus-like forms of the gametophytes of the ferns, lycopods, hepatics, and equisetums, in such manner as to constitute mycorrhizas, is well known.

A mycorrhiza is classed as ectotropic or endotropic, according to the manner in which the contact between the two plants is made. Ectotropic mycorrhizas are those in which the fungus forms a thick felt or sheath around the absorbing organs of the higher plant. This form is always made by the union of a fungus with true roots and never with stems, leaves, or prothallia. The sheathing mass of hyphæ may completely enclose the root, or it may be absent from the apical region of this member. In ectotropic forms the fungus does not actually gain entrance to any of the living cells of the higher plant, except in one or two families. The epidermal cells are papillose and touch each other at the base only, leaving wide interstices between their outer extremities, which are occupied by masses of hyphæ. As a result of this arrangement, the fungus appears to occupy the epidermal cells when a cross-section of the root is examined, but such is not the case except perhaps in *Pterospora*, *Monotropa*, *Hypopitys*, and *Sarcodes*, which I have recently examined as to this particular. *Populus* (poplar), *Quercus* (oak), and the conifers furnish examples of ectotropic mycorrhizas in which the fungus does not enter the epidermal cells.

In order to comprehend the other, or ectotropic type of mycorrhiza, it will be necessary to recall that the typical structure of roots is one in which the central portion of the organ is occupied by the stele, of which the fibro-vascular tissue is the principal component in bulk, and that this central cylinder is surrounded by a sheath composed of thin-walled cells of the cortex, outside of which lies the cortex proper, made up of ten to thirty layers of large parenchymatous cells. Covering the root is the epidermal system of one to three layers, with the outer walls of the outermost layer drawn out into tube-like extensions — the root hairs. The hyphæ of the fungus gain entrance to the root when quite young by pushing into it from the stem, or by piercing its tender epidermis. Once inside the root, it generally shows a differentiation into three regions. The hyphæ which enter the root push forward through the walls of the outer cortex toward the apex of the root, occupying one to three layers of cells with hyphæ which are fairly free from convolutions or enlargements. This portion of the body of the fungus may be termed the "vegetative mycelium." Numerous branches from the vegetative mycelium are sent into the middle region of the cortex, and these branches subdivide and pierce the thin walls of the cortex, so that they may occupy more than half of the cells. These internal branches appear to be most delicately chemotropic. In some plants they are attracted to the neighborhood of the nuclei, where they form large vacuolated sacs, termed "vesicles," or agglomerations of interwoven hyphæ, which doubtless serve as organs of interchange between these two plants, as will be described further on. In some instances the tips of the growing hyphæ are attracted to the plastids or to the centers of carbohydrate formation. The presence of the fungus with the organs of interchange generally sets up disturbances in the cells occupied, of which enlargement of volume, hyperchromatism, and sometimes fragmentation of the nucleus are the most noticeable. It is most notable, however, that the cells occupied by the vegetative mycelium undergo almost no variation from the normal characters of such organs. The cells of the middle portion of the cortex are generally very much enlarged, but this has been shown to be due to the character of the food material received rather than to the presence of the fungus. Branches of the vegetative mycelium pass outwardly through the epidermis, which may or may not traverse the root hairs, and after emergence into the soil they are numerously divided and extend to unknown distances in the soil. The mycorrhizal fungi of the gametophytes of the lower forms usually occupy the external or the lower layers of the thallus.

On account of the great elasticity of the various factors to be considered in the determination of the nature of the interchange which takes place in mycorrhizas, the final solution of the matter has been attended with great difficulty. The chief features of the metabolism of the uniting cells of the two organisms, the composition of the substratum, the anatomy of the higher plant, with special attention to degenerations, and the behavior of the two symbionts when grown alone or in pure culture must all be taken into account, and the consideration of all these points entails an enormous amount of observation, and no single mycorrhiza has yet been carried through all stages of the work. Our conclusions, which are fairly final as far as they extend, are therefore derived from evidence more or less fragmentary, so far as any single formation is concerned, yet is to be taken as fairly and justly supplementary.

So far as the various theories concerning mycorrhizal plants are concerned, it is to be said that Unger concluded that *Hypopitys* (*Monotropa hypopitys*) was parasitic on the roots of trees (1840). Pfeffer recognized the symbiotic relations of the members of a mycorrhiza in 1877, and this was demonstrated in

Hypopitys by Kamienski in 1881. Frank, in a series of studies published between 1885 and 1891, originated the theory that the higher plant is really parasitic upon the fungus, inducing it to grow on and in its roots, and finally yield its components to the higher plant, and he termed the arrangement a "fungus trap." Recently Janse has endeavored to prove that mycorrhizas sustain the same functions as leguminous tubercules (1896). The theory of Janse has been found to hold true of *Podocarpus* and the peronosporous fungus with which it forms mycorrhiza only, although it may be applicable to a few other special forms.

So far as the mutual dependence of the mycorrhizal symbionts upon each other is concerned, it may be stated, with a fair degree of certainty, that all of the fungi are capable of more or less extended periods of growth and endurance in the soil; though to what extent has not been ascertained. The higher plants which habitually form mycorrhizas are capable of development in sterilized soils, but according to Frank's experiments they do not attain the size of specimens united with fungi in the usual manner. This applies only to those species which are furnished with chlorophyl, and Frank's experiments were made with coniferous trees. The capacity of such chlorophylless forms as *Monotropa* and *Pterospora* for growth in sterile cultures has not yet been determined, though I hope to have some results within the coming season. It is pertinent to recall in this connection the fact that when spruces or other coniferous trees with mycorrhizal roots are cut down, the stump often forms a callus plate, which finally covers the entire injured surface, and it continues to live without forming leaves or other organs for constructing food from the air. This was thought by Frank to demonstrate that the living stump was parasitic on the fungus attached to the roots. This conclusion did not take into account the comparatively enormous amount of food stored in the roots of the tree, or of probable metabolic capacities of the protoplasm of the tree not possessed by the fungus.

Omitting the discussion of the anatomical details of the union of the two plants which form a mycorrhiza, I may say, in general, that we are justified in the following conclusions as to the relations of the symbionts; the higher plant furnishes a habitat for the vegetative mycelium of the fungus, and yields to it certain carbohydrate foods, principally starch and sugar. The fungus takes up humous compounds from the soil which are poor in oxygen, conducts them to the branches inside the body of the higher plant, and manufactures proteids which it in turn yields to the higher plant. The actual food substance may all come through the body of the fungus, yet it undergoes elaboration in the higher plant to forms which are very advantageous foods for the fungus, such as starch and sugar.

Ectotropic mycorrhizas are generally unmistakable, but in many instances it is difficult to determine whether the fungus inhabiting a root is parasitic or sustains symbiotic relations to the higher plant. Indeed it may do both at different stages of development perhaps. The chemical exchange between the two plants may balance at one time, while at another, one may receive a preponderance of benefit. Then again a mycorrhizal formation may enable the individual to obtain its food more economically, yet such easy nutrition may set up degenerations which will unfit the seed plant for living outside of the narrowest conditions, and which may result in the extinction of the species. *Pterospora* appears to be an example of such degeneration.

As a result of the investigations of the last decade, it is found that the habit of forming mycorrhizas is of very wide prevalence among the seed plants, ferns, lycopods, liverworts, and equisetums. This and the determination of the fact that all plants are capable of absorbing and using organic substances of some complexity of structure must be accounted among the most important results of research upon the nutrition of plants during the closing decade of the century.

So far as relationships are concerned, it may be said that the conifers, the orchids, the heaths, oaks, poplars, beeches are most thoroughly given over to the formation of mycorrhizas, and a few of the orchids have developed this adaptation to such extent that they have lost their chlorophyl and depend entirely upon the fungus for carbon compounds. The pine saps (*Mono-*

tropaceæ), formerly included with the heaths, comprise nine genera, *Allotropa*, *Monotropa*, *Pterospora*, *Hypopitys*, *Sarcodes*, *Newberrya*, *Schweinitzia*, *Pleuricospora*, and *Cheilotheca*, all of which have lost their chlorophyl and depend upon the fungi symbiotic with their roots for organic nutriment. All of these genera are inhabitants of the north temperate zone, arranged in a belt which circles the globe, but with the greater number of species and genera in western North America.

Hitherto all chlorophylless seed plants which were not parasitic have been classed as saprophytes, but in some recent publications I have been able to show that this term may be applied only to one species of seed plants, *Wullschlægelia aphylla*. All other so-called saprophytes are found to be symbiotic with fungi in such manner as to form mycorrhizas. The tendency to form mycorrhizas is most marked among species living in humous soils. The percentage of the flora with the mycorrhizal habit increases from the arctic circle to the equator as a consequence of this fact. Hoveler describes mycorrhizas on 24 of the 72 species, native and cultivated, which he examined in northern Germany. Schlicht found 70 of the 105 species native to northern Europe to be provided with mycorrhizal adaptations. The writer found 13 of the 15 selected species growing in the United States to be mycorrhizal. Janse found mycorrhizas on 69 of the 75 species growing in Java, which he examined. Wahrlich examined 500 species of orchids, chiefly from the tropics, cultivated in the greenhouses at Moscow, all of which he reports as being furnished with mycorrhizas.

The identity of the greater number of fungi which enter into mycorrhizal combinations is unknown. Present information leads us to believe that each species of the higher plants forms mycorrhizas with one, or at most two or three fungi only in the different parts of its range. On the other hand, it is fairly probable that one species of fungus is capable of entering into such combinations with several of the higher plants.

Tulasne noted the fact that *Elaphomyces* forms the ectotropic mycorrhizas on the roots of pines, to which Boudier attributed a parasitic relation. Reissek determined the fungus on *Platanthera bifolia* as *Fusisporium endorhizum*. Schacht found that the fungus in the roots of *Limodorum* developed reproductive organs similar to *Eurotium*. Treub and Bruchmann conclude that a *Pythium* forms the symbiosis with the prothallium of *Lycopodium*. Bruns found that *Polysaccum* formed coatings on the roots of pines. Woronin identified the mycelia on conifers, willows, poplars, hazelnut, birches, and grasses as belonging to the *Boleti*. Rees determined the fungus on certain conifers as an *Elaphomyces*. Janse named the one on *Celtis*, *Celtidia duplicispora*, to be included with the *Tuberaceæ*. Recently Rees and Fisch have examined the mycelia formed by *E. granulatus* and *E. variegatus* on the roots of the forest trees, and conclude that the relation is not a parasitic one, but constitutes a mycorrhiza. Noack made some attempts to form mycorrhizas by experimental methods in 1887–1889, and found that *Geaster fimbriatus* and *G. fornicatus* form mycorrhizas with the roots of conifers; *Agaricus terreus* with beeches and firs; *Lactarius piperatus* with *Fagus sylvatica* and *Quercus pedunculata*; *Cortinarius callisteus* and *C. coerulescens* with beeches; *C. fulmineus* with oaks. Wahrlich determined the fungus of the mycorrhiza of *Vanda tricolor* as *Nectria goroschankiana*, and that of *Vanda suavis* as *N. vanda*. Lendner made some spore cultures of the fungi of *Platanthera* and *Vanda* in 1895, and his observations confirm the results of Wahrlich. Chodat and Lendner found that the fungus of *Listera cordata* resembles *Nectria* as originally described by Wahrlich on Vanda. Jennings and Hanna conclude that the fungus symbiotic with *Corallorhiza innata* is a "hymenomycete and commonly an agaric." *Clitocybe infundibuliformis* was found attached to the coralloid formations in one instance, and *Hysterangium stoloniferum* in another — indicative of their identity with the fungal symbiont. Nobbe and Hiltner observed the peronosporous fungus in the mycorrhiza of *Podocarpus*, and the writer has found reproductive bodies resembling *Penicillium* on the mycorrhizal roots of *Pterospora*. Jeffreys regards the fungus of the gametophyte of *Botrychium* as being intermediate between a *Completoria* and a *Pythium*. These and a few other examples pointed out by Sarauw in his review of the subject in 1893 (*Rodsymbiose og Mykorrhizer*, Copenhagen, 1893) comprise our information upon this phase of the subject.

All known species of mycorrhizal fungi may therefore be included in the *Oömycetes*, *Pyrenomycetes*, *Hymenomycetes*, and *Gasteromycetes*. The independent culture and identification of these fungi offers a most alluring field to the mycologist, and the exploitation of this work will doubtless result in the setting up of scores of new species, the erection of genera, the addition of new chapters to the life history of polymorphic forms, and the furtherance of our knowledge of the morphology of this great group of organisms, all of which will be of vital importance in the determination of the essential features of the general physiological value of mycorrhizas and the humus relations of the higher forms.

The widespread occurrence and distribution of mycorrhizas render this adaptation of high importance in the nutrition of the greater number of the perennial seed plants. At the present time no reports are at hand of any attempt to use our information on this subject in horticultural work, or in the cultivation of economic forms, although I confidently predict that it will ultimately be found of high value in the transplantation and growth of woody plants, shrubs, and trees cultivated for fruit, foliage, and other products ; a forecast easily comprehended when we glance at the vast agricultural importance of the results of our study of the leguminous tubercles, which are in fact a special form of mycorrhizas.

Since the above lecture was written, Bernatsky's paper has come to hand, in which he transfers the species of *Nectria* seen by Wahrlich in *Vanda tricolor* to *Hypomyces*, and finds that *H. Vandæ* (Wahrl.) *Bernts.* also unites with *Vanilla aromatica* and *Psilotum triquetrum* to form mycorrhizas, and that *Hypomyces Psiloti Bernts.* is the fungal symbiont in the mycorrhizas of *Psilotum*. (J. Bernatsky. Adatok az endotroph mykorhizak ismeretehez. Termeszetrajzi Fuzetek. 22 ; 88. 1899.)

FIFTH LECTURE.

INSTINCT.

EDWARD THORNDIKE.

I HAVE first of all a request to make of the reader. It is that, in receiving and estimating what I say, he temporarily discard the definitions or formulæ for psychological phenomena now in his mind. Many of the discussions and quarrels of comparative psychology are about mere words, and are therefore fruitless. We can avoid all such if for the time being I am allowed to *name* the phenomena, the facts about which we are to think, as I please. I hope to make clear what facts I am referring to in every case, and you will be at liberty to rename them after your own preferences as soon as we part company.

The facts to which I refer by the words "instinctive reactions" or "instinctive activities" or "instincts" are any activities which do not have to be learned, which the animal is capable of without experience. Let us not mind whether the act be accompanied by consciousness or not, whether it represent the inheritance of some ability acquired by the animal's ancestors or not, whether it involve emotional feeling or not. I shall, for the sake of keeping in line with customary usage, deal with such instinctive activities as physiology generally leaves out, *e.g.*, instinctive fears, food preferences, motor control in running, jumping, flying, etc., though breathing, defecation, sleeping, etc., really deserve treatment of just the same sort as these more complex activities. Under instincts, then, let us study all the abilities to respond to different situations (more particularly certain complicated external situations) which the animal has to start with, has apart from parental or self tuition. An instinctive act, then, will be the opposite of an act due to experience. The sum of an animal's instinctive abilities plus the habits taught him by his life struggle will be the total of his store of ability.

It is clear that what an animal has to start with, due to the organic structure it inherits, is a matter of prime importance in the case of any animal whose consciously directed activities you are studying. If we do not know its instinctive equipment, we are likely to credit it with intelligent thinking for doing something it really could n't have helped doing. Suppose I say to you, "I have an intelligent chick who has learned so to adapt his movements in the water that he can swim to shore," and tossing him into the water, demonstrate that fact to you. It is probable that many a one would say, "How remarkable! Do you suppose he reasoned out the way to act? How *did* he learn to do it?" The real fact would be that he did not *learn* to do it at all; that all chicks react to water by swimming out of it the very first time they ever get in it; that chicks swim instinctively. How long error may persist about any animal activity is well shown by the recency of our knowledge that walking is instinctive in the human infant. So the first task of comparative psychology is to find out the instinctive equipment of any animal studied. Instincts are, however, well worth study for their own sake. An instinctive fear of a certain enemy may be as truly useful to an animal as sharp teeth or protective coloration. Instincts are the expressions of structures and functions of the nervous system, and are as real and as important matters for the biologist as are bones and blood vessels.

It is outside the province of this lecture to enumerate or describe the particular instincts of any group of animals, but we should note that in spite of the tremendous number of instincts that have been observed, the story has not been half told. A rich field awaits the investigator. The humble chicken has been under everybody's observation, and has been specially studied by Spalding, Eimer, Preyer, Lloyd Morgan, and others, yet I was able to record the following additional instinctive activities: swimming to shore when thrown into the water, fighting (observed as early as the sixth day), appropriate reactions to distance in jumping down from heights, avoidance of open places, reacting to honey-bees by seizing, knocking them against the ground, and then eating them (see *Psychological Review*, May, 1899).

Turning to our proper task, the statement of some characteristics of instinctive activities in general, we should first recall the familiar fact that to say that a certain ability or tendency is in an animal apart from teaching or experience, is born in him, is not to say that at birth he possesses it or that all through life he keeps it. Scratching the ground in the case of the chick, walking in the case of the human infant, are obvious examples of true instincts which yet do not appear for some time after birth. The instinct to follow and the instinctive avoidance of loneliness which the chick manifests so markedly in the earliest days of his life disappear to a large extent if given no chance to harden into habits. Instincts, that is, may be *delayed* and may be *transitory.* Care must always be taken by the student, however, not to interpret as delayed instincts cases where the reason for delayed activity is really lack of strength in some organ, or as transitory instincts cases where the reason for loss of the activity is loss of strength or inhibition of the activity by unpleasant consequences.

Perhaps the most important general statement one can make about instinctive reactions is that they are often indefinite and inexact. The same situation may be reacted to differently by different individuals of a species, or by the same individual on different occasions. The Peckhams (Wisconsin Geological Survey, *Bulletin No. 2*) have shown decisively this inexactness, this vagueness of reaction in the case of the nest-building and stinging habits of the solitary wasps. Among vertebrates one sees it almost everywhere. If you slam a door in a room where you have half a dozen chicks, one may run and crouch for twenty seconds, another may squat where he is for an equal time, another may also chirp, while the others may keep on feeding undisturbed. The old theory of instinct, that it was a sort of God-given power bestowed on animals to make up for their lack of reason, which in mysterious ways directed the animal's footsteps and aroused in him by direct inspiration the appropriate act in any set of circumstances, left us an unfortunate legacy in the shape of a tendency to expect an accuracy and infallibility and unchangeableness in animals' reactions such as supernatural inspiration might well give, but which are usually signs of death in the natural world. The common human interest in the marvelous and unusual coöperated with this tendency by selecting for observation such instinctive activities as web-spinning, honeycomb construction, etc., and neglecting the more ordinary activities. Thus arose such amusing expressions as "Failure of instinct." Furthermore, so long as there survived vestiges of this teleological notion of an entity, "instinct" which fitted acts to situations so as to get desirable results, there was the less interest in the real intermediary between situation and act, namely, the nervous system, and less likelihood of men looking for the variations in response to be expected in the results of the activity of any living organ.

The recognition of the vague in instinctive activity not only brings such activity into line with other biological phenomena, and lends a healthier tone to investigation, but also provides a useful warning to the observer. The naturalist who studies a single case of such activity is almost sure to be misled. "In a multitude of witnesses there is strength."

The utility in the struggle for existence of an animal's equipment of instinctive reactions is much increased by reason of their ability to harden into habits. It is a wide if not a general law that any act which has been performed in a certain situation and resulted in pleasure, or even indifferently, is the more likely to be performed again in that same situation, the reverse happening to any act resulting in discomfort.

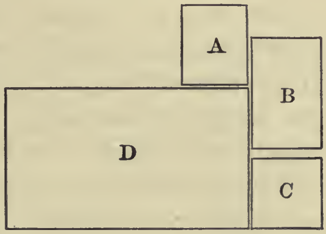
The general manifestations of this law will be dealt with in a later lecture, but its influence on instinctive reactions deserves mention here. First of all, transitory instincts may gain thereby equal value with permanent ones.

The chick that has followed the mother-hen for six or eight days does not thenceforth need any instinctive impulse to follow, for the act has become habitual and will remain in the absence of any such impulse. Again vague responses may be hardened into more definite and more successful forms. A cat with a general instinct to jump at small birds might with practice jump straighter and more quickly because the quick, straight jumps would result in the pleasure of capture. One is likely, however, to be misled if he argues from the presupposition that acts always tend to assume a "perfect" form. If the animal gets along very well with its instinct still "imperfect," there may be no change. Lloyd Morgan, for instance, has chosen a dubious example of perfecting through habit in the seizing of bits of food by chicks. They often do fail to seize in their first experiences, as he observed, but they often, perhaps just as often, fail even after long experience. I took nine chickens from 10 *to* 14 *days old* and placed them one at a time on a level surface over which were scattered bits of cracked wheat (the food they had been eating in this same way for a week) and watched their pecking. Out of 214 objects pecked at, 159 were seized, 55 *were not.* Out of the 159, *only* 116 were seized on the first peck, 25 on the second, 16 on the third, and 2 on the fourth. This is far from a perfect record.

In the growth of the chick's discrimination between objects as food we find a sure manifestation of our law. The chick instinctively pecks at all sorts of objects of suitable size, *e.g.*, tacks, match ends, printed letters, the eyes and toes of his mates, his own excrement, etc. The pecks at bits of food and small stones bring satisfaction, and the chick that when first confronted by the situation, "grain of wheat, match end, and excrement," was as likely to peck at one as another, becomes a chick who almost inevitably pecks at the wheat. Thus the vague instinctive response may be educated into a lot of definite food preferences and avoidances. Not only thus directly, but also in indirect and complex ways, instincts may furnish the foundation of habits or, as we may better call them, associations; associations, that is, between certain situations or circumstances and certain acts. A young animal instinctively follows or keeps near its parent and thereby forms associations which later will lead him to go independently to certain feeding grounds, to eat certain sorts of animals or plants, to run away from certain enemies, to sleep in certain lairs. A kitten when confined in a cage reacts instinctively with squeezings, clawings, bitings, etc., some one of which may happen to open a thumb-latch on the door and give it freedom. The pleasure consequent may so stamp in that particular act, in connection with that situation, that after enough experiences the animal will, when put in that situation, manifest nothing of all the instinctive activities first observed save the one particular poke at the thumb-piece. Its activity now would seem far enough from instinct to many people, but it is really a consequence of instinctive activity. It has come to neglect the unsuccessful squeezings and bitings, to choose the successful clawing, in just the same way that the chick comes to neglect the peckings at excrement and match ends, and choose the food.

I have already implied that instinctive activities may be inhibited just as truly as they may be confirmed and reinforced. If the conditions in which an animal lives become such that an instinctive act brings discomfort, that activity tends to disappear; or if the animal has, prior to the appearance of a certain instinct, learned to meet otherwise a situation which would normally call forth the instinct, he may continue to meet it in that rather than the instinctive way. It would even be fairly reasonable to interpret the transitory instincts as instincts which were inhibited by mere lack of exercise. A convenient account of the inhibition of instincts may be found in James's *Psychology*, Vol. II, pp. 394–397. I may quote in addition examples of inhibition (1) by virtue of the previous formation of a habit, (2) by (1) plus actual abolition through resulting discomfort.

An instance of the former sort is found in the history of a cat which learns to pull a loop and so escape from a box whose top is covered by a board nailed over it. If, after enough trials, you remove a piece of the board covering the box, the cat, when put in, will still pull the loop instead of crawling out through the opening thus made. But, at any time, if she happens to notice the hole, she *may* make use of it. An instance of the second sort is that of a chick which has been put on a box with a wire screen at its edge, preventing it from jumping directly down, as it would instinctively do, and forcing it to jump to another box on one side of it and thence down. In the experiments which I made, the chick was prevented by a second screen from jumping directly from the second box also. That is, if in the accompanying figure, A is a box 34 inches high, B a box 25 inches high, C a box 16 inches high, and D the pen with the food and other chicks, the subject had to go A–B–C–D. The chick tried at first to get through the screen, pecked at it and ran up and down along it, looking at the chicks below and seeking for a hole to get through. Finally it jumped to B and, after a similar process, to C. After enough trials it forms the habit and when put on A goes immediately to B, then to C and down. Now if, after 75 or 80 trials, you take away the screens, giving the chick a free chance to go to D from either A or B, and then put it on A, the following phenomenon appears. The chick goes up to the edge, looks over, walks up and down it for a while, still looking down at the chicks below, and then goes and jumps to B as habit has taught it to do. The same actions take place on B. No matter how clearly the chick sees the chance to jump to D, it does not do so. The impulse has been truly inhibited. It is not the mere habit of going the other way, but the impossibility of going *that* way. In one case I observed a chick in which the instinct was all but, yet not quite, inhibited. When tried without the screen, it went up to the edge to look over *nine times*, and at last, after seven minutes, did jump straight down. — *Animal Intelligence, an Experimental Study*, etc., pp. 99–100. New York, 1898.



It remains to consider the so-called "perversions" of instinct, that is, the cases where an instinctive activity appears in response to a situation which it does not fit, or does not ordinarily go with. The cat that nurses rats or puppies, the pigeon that capers before a bottle, are stock examples. Most of such are due to the essential indefiniteness of the instincts in question. The cat does not have an impulse to nurse a particular, definite thing, to wit, a young kitten, and to abhor all else as subjects for nursing, and if her own young are only taken away and other young nursing things happen to be around, they may call forth the activity quite as well. Other "perversions" may be brought about by naturally or artificially caused inhibitions. There may also, of course, be in animals, as there may be in men, abnormalities in the nervous system which may occasion abnormal instinctive manifestations. These would need separate treatment. The interest of the ordinary "perversions" is simply their witness, first, to the subordination of instincts to the laws of nervous activity in general, *e.g.*, variation, association, inhibition, and, secondly, to the coarse, roughhewn nature of many instincts.

So much for the important general facts about how animals meet situations apart from experience. Before entering on the tasks of justifying the definition of instinct, which I asked you to accept provisionally, and of saying somewhat about the origin and evolution of instincts, I wish to take this opportunity to advocate the study of instinctive activities by students of elementary biology. Their observation requires no knowledge of psychological terminology, no ability to make psychological interpretations. The observer has simply to answer the question, "How does an animal react to a certain situation the first time he is in it?" The demonstration of every matter discussed so far in this paper may be effected at practically no expense in any quiet place where the student can find twenty square feet of space in which to keep a few chicks. Such study will serve as a partial corrective to the tendency to make biology a study, not of life, but of form. It may give the student a taste of and for fact in this field of biology, which will keep him from getting lost when he meets with the speculations about instinct. It is, moreover, worth while for its own sake.

Returning now to the question of definition, it is clear that any one has a logical right to name things to suit himself and to determine the particular things which he shall denote by a certain word at his own sweet will, so long as he is clear and consistent and does not reason from any other meaning of the word than that he has given to it. One might, for example, in a study of fission call Paramœcium "Hylobates McKinleyensis" and commit no logical error, provided his figures showed clearly the animal he meant to talk about, and provided he did not use the name to argue, for instance, that we might, having found fission in Hylobates McKinleyensis, expect it in the forms ordinarily called "Hylobates." Names are not facts, and quarrels about them are generally quarrels about rhetorical expediency. So if any one chooses to call instinctive only such unlearned acts as have consciousness accompanying them, leaving other such acts the name "reflex action" and lumping in still other such acts under the words "processes of respiration, digestion," etc., I, personally, have no theoretical objection. It seems to me *practically* unwise to separate the discussion of unlearned activities with consciousness from that of unlearned activities without consciousness, so long as you are thinking about the external form of the act; and I have even chosen to so define the word that even digestion and respiration could be studied under the name. There is really no need for *any* name and no cause for worry if we cannot invent any one definition which will consistently apply to all the facts we treat. For what is important is concrete information about particular facts. My use of a name or definition is to point to certain actual facts, and I trust that I have made clear by my illustrations to what sort of facts my statements refer. To one who should say, "By your definition the knee-jerk or the formation of saliva is an instinct, for they are 'unlearned reactions,' — may even be considered reactions to a 'complex external situation,' — yet your statements about the formation of habits and inhibition are not true of them," I should reply, "I plead guilty, though I did describe further my facts as those which 'physiology generally leaves out,' but you are a more stupid reader than I expected to address."

The fact is that unlearned activities range from direct physical and chemical activities of single cells to most complicated activities involving the nervous system; that the series seems to be continuous; that if you try to separate off one lot "instincts," another lot "reflexes," another lot "non-nervous reactions," you find your classes running into each other. It is because of my confidence in this continuity of nature that I used my definition only to point to what I was talking about —

not to describe authoritatively the attributes of a fixed class of facts. Practically it seemed worth while to bring out this continuity in the definition.

This rather tiresome explanation serves an additional purpose by introducing us to a real question—the question of the origin of instincts. For some thinkers have cheerfully begged this question, by defining instincts as inherited habits, and by studying those unlearned abilities which looked like inherited habits, out of connection with all other unlearned abilities. In his lecture on "Animal Behavior" (Woods Holl *Biological Lectures*, pp. 285–338, 1898) Prof. C. O. Whitman has, in opposition to these thinkers, defended with great care and force the theory that such unlearned abilities as the spider's web-spinning, chick's scratching, dog's pointing, etc., — such activities, in short, as have been discussed in this paper, — (1) have the same origin as the unlearned activities of reflex action, digestion, circulation, etc., (2) are due, in fact, to organic features, which again (3) are due to germ variations, and that (4), therefore, we should look for and expect to find, so far as traces have been left for us to follow, as continuous a development of such activities, as true an evolution of "instincts" as of organs.

All that I have to say about Professor Whitman's first two contentions is that they seem to me so true that I cannot understand any one's doubting them. As one runs through animals' unlearned activities, from those most to those least like products of learning, he can nowhere stop and say, "hitherto inherited habits, from hence chance variations." These first two contentions would remain true whatever might happen to the third. As to the third, I can only add my mite to the evidence. The failure of chicks to avoid their own excrement and their ability to swim seem hard to explain on Lamarckian principles. For the avoidance of excrement must have been formed as a habit in every individual for many generations, while the swimming instinct has been unused for even more perhaps. Again, the vagueness of response discovered the more we study animals' unlearned activities is just what one would expect if these responses were due to germ changes selected by reason of their success in procuring survival, while it is not what we would expect from the inheritance of habits acquired in reference to definite objects. If the instinct to follow in chicks came from the habit of following (acquired, of course, with a hen as the object followed), we would expect it to require as its object something at least like a hen. As a matter of fact it does not.

Professor Whitman's fourth contention, that, since instinctive activities are the results of gradual development, they should be, not merely enumerated, described, and explained as to their utility, but also explained as to their development and relationships, comes as a timely piece of advice. Even if students of instinct should never succeed in working out the genealogy of one instinct out of a hundred, the genetic method of study would be valuable by preventing mythologizing and reminding the student that instinctive activities are expressions of organic structure as truly as are the activities of digestion or excretion. And, in closing this lecture, I wish to give some samples of development among instincts. Professor Whitman has traced the ancestry of some particular acts. Let us look at some instinctive activities which persist, with modifications of course, over a wide range of forms, which correspond in a way to the notochord, or brain-eye, or arthropod appendages among physical organs. The frog, lizard, chick, and cat all react to irritation of the head by scratching with the hind leg with a quick, repetitive motion that is startlingly alike in the last three. Here we have an instinct which apparently ranges nearly over a subkingdom. In the primates it is modified, the monkeys (at least some of them) using *either* hind or front limb for the purpose, while man uses only the front. Distaste at confinement is another widely prevalent instinct, which may be a foundation-stone laid by germinal variation once for all. The instinct to follow is another. It might be that the gregarious instincts originated as exaggerations of it by the selection of individuals in whom the impulse to follow varied in the line of greater intensity.

SIXTH LECTURE.

THE ASSOCIATIVE PROCESSES IN ANIMALS.

EDWARD THORNDIKE.

In the previous lecture we studied the general nature of those reactions which animals make to various situations instinctively, or apart from experience. It is obvious that with many animals, in many situations, acts are observable which cannot be so explained. The cat that comes when we call "Kitty, kitty," is not provided by her organic inheritance with any tendency to respond to the situation, "hearing the sound *kitty, kitty*," by the act, "running toward the source of that sound." The ten-days-old chick that, on coming out from the brooder, turns round a corner and goes straight to the dish of water kept always in a certain place, is not guided by any innate tendency to meet the situation, "feeling thirsty while in brooder," by that particular act. So, too, with the dog that sneezes when you say, "Sneeze, Bowser!" the chick that avoids pecking at excrement, and with millions of animals performing all sorts of acts. We ordinarily distinguish such activities from those purely instinctive by saying that the animal has *learned* them. They are the results, not of organic inheritance, but of individual experience. The object of the present lecture will be to explain such activities, to show at least one of the ways in which animals learn or profit by experience.

Some hints of how this happens were given in our discussion of how instincts led to habits and how they were inhibited. But the matter is so important that I may be pardoned for beginning at the beginning. Let us watch some animals as they learn to meet certain situations in appropriate ways. If we make a pen, as shown in Fig. 1, and put a chick, say six days old, in at *A*, it is confronted by a situation which is, briefly, "the sense-impression or feeling of the confining surfaces, an uncomfortable feeling due to the absence of other chicks and of food, and perhaps the sense-impressions of the chirping of the chicks outside." It reacts to this situation by running around, making loud sounds, and jumping at the walls. When it jumps at the walls, it has uncomfortable feelings of effort; when it runs to *B*, or *C*, or *D*, it has a continuation of the feelings of the situation just described; when it runs to *E*, it gets out, feels the pleasure of being with the other chicks, of the taste of food, of being in its usual habitat. If from time to time you put it in again, you find that it jumps and runs to *B*, *C*, and *D* less and less often, until finally its only act is to run to *D*, *E*, and out. It has, to use technical psychological terms, formed an association between the sense-impression or situation due to its presence at *A* and the act of going to *E*. In common language it has *learned* to go to *E* when put at *A* — has learned the way out. The decrease in the useless runnings and jumping and standing still finds a representative in the decreasing amount of time taken by the chick to escape. The two chicks that formed this particular association, for example, averaged one about three and the other about four minutes for their first five trials, but came finally to escape invariably within five or six seconds.

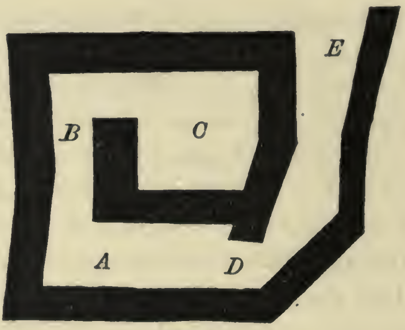


FIG. 1.

The following schemes represent the animal's behavior (1) during an early trial and (2) after the association has been fully formed — after it has learned perfectly the way out.

(1)

SITUATION.	IMPULSES.	ACTS.	RESULTING FEELINGS.
As described above.	To chirp, etc. To jump at various places. To run to *B*. " " " *C*. " " " *D*.	Corresponding to impulses.	Continuation of situation. Fatigue.
	" " " *E*.		Pleasure of company. " " food. " " surroundings.

(2)

SITUATION.	IMPULSES.	ACTS.	RESULTING FEELINGS.
Same as (1).	To run to *E*.	Corresponding to impulse.	Pleasurable as above.

A graphic representation of the progress from an early trial to a trial after the association has been fully formed is given in the following figures, in which the dotted lines represent the path taken by a turtle in his fifth (Fig. 2) and fiftieth (Fig. 3) experiences in learning the way from *A* to his nest. The straight lines represent walls of boards. Besides the useless traveling, there were, in the fifth trial, useless stoppings. The time taken to reach the nest in the fifth trial was seven minutes; in the fiftieth, thirty-five seconds. The figures represent typical early and late trials, chosen from a number of experiments on different individuals in different situations, carried on at Woods Holl by Mr. R. M. Yerkes, of Harvard University, to whom I am indebted for permission to use these figures.

Now the process of learning here consists of the selection, from among a number, of a certain impulse and act in connection with a certain situation. And our first business is to discover the cause of that selection. The result of such discovery was dogmatically stated in the previous lecture: "Any impulse to an act which, in a given situation, leads to pleasurable feelings, tends to be connected more firmly with that situation; and any impulse to an act which, in a given situation, leads to discomfort, tends to become weakened in connection with that situation." I say *tends* because the pleasurable feelings must follow the act within certain limits of time — must be important enough to outweigh possible instinctive opposition to the act, or possible discomfort in its performance. Any one may demonstrate to himself the truth of this law over a wide range of animal life by observing the nature of the acts selected and of those eliminated by experience. He will find that the com-

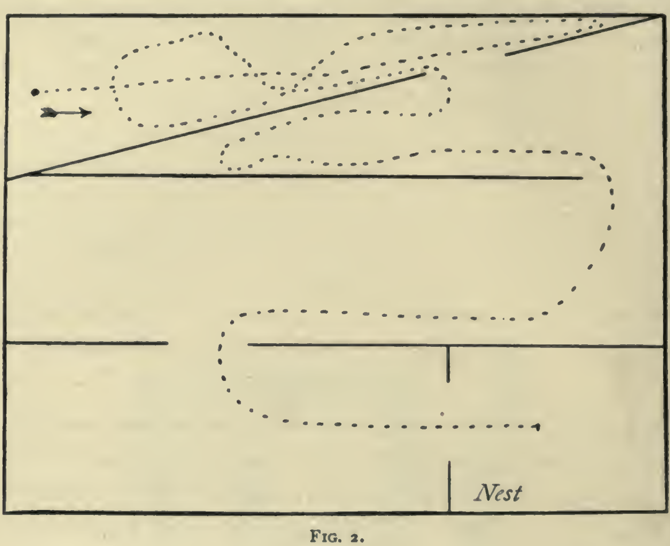


FIG. 2.

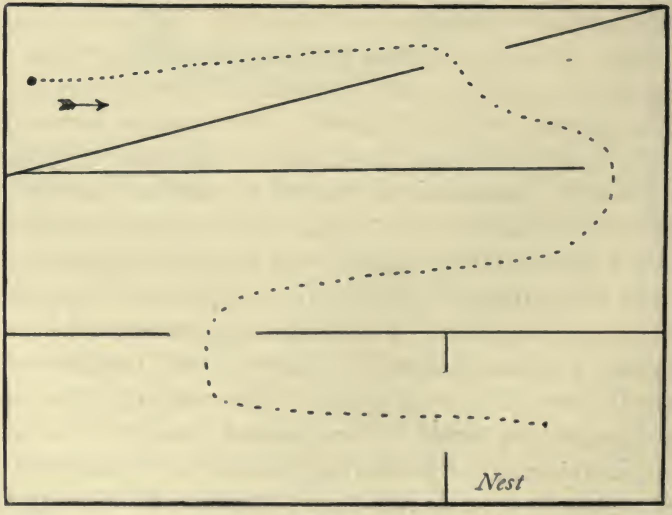


FIG. 3.

mon element of the former is resultant satisfaction, and of the latter the opposite. Later on it will be worth our while to examine more carefully the way in which pleasurable and painful consequences respectively stamp in and stamp out the impulses which lead to them. For the present we had better return to actual specimens of animal behavior.

In the first place, the selection may be of one impulse and act from only one. If the chick had happened to go to *E* the first thing he did, the resulting satisfaction might have so stamped in that impulse that on the second, third, and later trials he would never have done otherwise. If accident or instinct furnishes the right impulse, it will naturally be confirmed just as readily as if picked out by its success from any number of inappropriate ones.

It will be well now to examine a more ambitious performance than the mere discovery of the proper path by a chick. If we take a box twenty by fifteen by twelve inches, replace its cover and front side by bars an inch apart, and make in this front side a door arranged so as to fall open when a wooden button inside is turned from a vertical to a horizontal position, we shall have means to observe such. A kitten, three to six months old, if put in this box when hungry, a bit of fish being left outside, reacts as follows[1]: It tries to squeeze through between the bars, claws at the bars and at loose things in and out of the box, reaches its paws out between the bars, and bites at its confining walls. Some one of all these promiscuous clawings, squeezings, and bitings turns round the wooden button, and the kitten gains freedom and food. By repeating the experience again and again, the animal gradually comes to omit all the useless clawings, etc., and to manifest only the particular impulse (*e.g.*, to claw hard at the top of the button with the paw, or to push against one side of it with the nose) which has resulted successfully. It turns the button round without delay whenever put in the box. It has formed an association between the situation, "confinement in a box of a certain appearance," and the impulse to the act of clawing at a certain part of that box in a certain

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[1] Confinement alone, apart from hunger, causes similar reactions, though not so pronounced.

definite way. Popularly speaking, it has learned to open a door by turning a button. To the uninitiated observer the behavior of the six kittens that thus freed themselves from such a box would seem wonderful and quite unlike their ordinary accomplishments of finding their way to their food, beds, etc., but the reader will realize that the activity is of just the same sort as that displayed by the chick in the pen. A certain situation arouses, by virtue of accident or, more often, instinctive equipment, certain impulses and corresponding acts. One of these happens to be an act appropriate to secure freedom. It is stamped in in connection with that situation. Here the act is "clawing at a certain spot" instead of "running to *E*," and is selected from a far greater number of useless acts.

In the examples so far given there is a certain congruity between the impulse associated with the situation and the result. The act which lets the cat out is hit upon by the cat while trying to get out, and is, so to speak, a likely means of release. But there need be no such congruity between act and result. If we confine a cat and open the door and let it out to get food only when it scratches itself, we shall, after enough trials, find the cat scratching itself the moment it is put into the box. Yet in the first trials it did not scratch itself in order to get out, or indeed until after it had given up the unavailing clawings, etc., and stopped to rest. The association is formed in the same way with such an "unlikely" or incongruous impulse as that to scratch, or lick, or, in the case of chicks, to peck at the wing to dress it.

The examples chosen so far show the animal forming a single association, but such may be combined into series. For instance, a chick learns to get out of a pen by climbing up an inclined plane. You then so arrange a second pen that the chick can, say by walking up a slat and through a hole in the wall, get from it into pen No. 1. After a number of trials the chick will, when put in pen No. 2, go at once to pen No. 1 and thence out. You then arrange a third pen so that the chick, by forming another association, can get from it to pen No. 2, and so on. In such a series of associations the "act" of one brings the animal into the "situation" of the next, thus arousing its act, and so on to the end. Three chicks thus learned to go through a sort of long labyrinth without mistakes, the "learning" representing twenty-three associations.

The next matter to examine is the set of feelings in the animal which we have called "feelings of the situation," or, more simply, "the situation." The important thing about them is their vagueness, indefiniteness. The kitten did not have in mind every bar of the box confining it, and feel the impulse to turn the button in connection with such a precise sense-impression. It felt the whole environment in an extremely hazy way, and would still have turned the button if you had planed off one-quarter of each bar, or painted the button, or put the box in a different place, or sprinkled the bottom with sawdust, though any of these acts would have vastly altered the situation from our point of view. Cats that climbed up a certain screen when I whistled three times and said, "I must feed those cats," would climb up just as surely if I whistled only or spoke the words only, or even used any short sentence with the same voice and manner. If I took the button off in the box referred to above, the animals that had formed the association would often claw as before, though the (to us) essential part of the situation was absent. Cats that had often obtained food in consequence of being made to go into a certain box, from which they liberated themselves by pulling down a wire loop, would go in and pull the loop and then come out, though no food was there and the door was open all the time. All these facts witness to the vague, indefinite character of the animal's feelings of any of these situations, and to the consequent fact that much may be added to or subtracted from the external situation without essentially modifying the animal's reaction to it. Thus the kitten that in the first place responded with the act of approach to the situation, "smell and sight of milk in a certain dish," might later respond similarly to the sight of the dish, though it was empty; thus the kitten that in the first place responded to the situation, "sight or smell of food plus hearing of certain sounds," might later respond similarly to the sounds alone, might come, that is, at our call. It is by virtue of this power of a single element in a situation to set off the reaction associated with the vague total situation, that horses are trained to stop at "Whoa!" and to turn at the slightest pull of the rein, etc. It is in any particular case a concrete problem how far you can change the external situation and still secure the reaction associated with it. By such *gradual* modification as we make use of in training domestic animals you can of course change it without limit. For our present purposes we need not bother with any instances of this problem, remembering only that the animal does not react to a well-defined, hard-and-fast feeling or notion of its surroundings, nor with an immutable act, much less does it react to a well-defined feeling of the essentials (for its purpose) in the situation, and that therein lies the explanation of a host of animal activities.

So much for the feelings which are the first element in the association. Somewhat must now be said about the "impulses." I have all along spoken of impulse and act, because for various reasons out of place in this discussion I believe that there is in one of these associations, besides the feeling of the situation and the resulting act, a feeling preparatory to that act, such as we ourselves feel in connection with any non-automatic muscular performance. Such a feeling is meant by the word "impulse." It does not, in my usage, mean "motive" or "desire," or even the idea of the act to be performed, but merely the, in our own case, rather evanescent feeling of doing. Whoever wishes may discard this feeling from consideration, and substitute for "impulse and act" the word "act" alone, and refer what is said in the rest of this paragraph about the former to the latter. In the cases so far considered, the causes of the impulses and acts manifested at the first experience of a certain situation have been, apart from accident, the instinctive tendencies of the animals in question. That was because the particular situations chosen (the pen for the chick, box for the kitten, etc.) were situations for which the animals' previous experiences had given them no preparation. But suppose us to take the kitten that had formed an association between confinement in that box and the act of clawing at a certain place and put it in another box. We would witness less biting and squeezing and more clawing than we did in our previous experiment. For its previous experience would have strengthened the association between clawing and confinement, or at least weakened it less than it had weakened the impulses to bite and squeeze. That is, in any situation the impulses and acts which appear are due not only to the animal's instinctive tendencies, but also to any modifications of them which have been wrought by associations already formed. The question of how an animal will act in any situation may thus require, for correct prophecy, knowledge not only of its inborn constitution, but also of its entire previous history.

Let us now return to the simple cases of animal behavior with which we started and interpret them in accordance with the facts we have found in our observation of the formation of associations under test conditions. The cat's coming when we call, "Kitty, kitty," we have already explained as a case of an act associated with a certain total situation first (because, of course, the act brought the pleasure of eating), then with one element of that situation. The chick coming out of the brooder, turning round a corner, and going straight to the dish of water, leads back to a history somewhat like this:

Situation.	Impulses.	Consequent Acts.
Being in yard. Feeling thirsty.	To walk around and to peck at things, *e.g.*, at stones, seeds, worms, specks of all sorts, *e.g.*,	
	specks of dirt in the water of water dish, the edge of the dish.	These lead to the further acts of drinking.

The last part of this piece of experience leads by repetition to the formation of an association between the situation, (*A*) "presence near dish when thirsty," and the act, "drinking." But as soon as it is partly formed there is also being formed an association between the situation, (*B*) "presence near brooder when thirsty," and the act, "walking around the corner"; for whenever in that situation the chick by accident does stroll around the corner, it comes to feel situation *A*, and so to be led to the pleasurable drinking. The chick's conduct (and other conduct like it), then, is the result of several associations, the act of one of which brings the animal into the situation of the next. Of such serial associations the chick in the labyrinth showed us a good example. The dog sneezes at our command, because in teaching him we held his nose so as to compel him to sneeze at the same time that we gave the command. The act of sneezing thus became associated with a total situation, including the pressure, etc., at the nostrils and the sound of our command, and was later on committed when the latter part only of the situation was present.

The behavior of animals under test conditions has, then, shown us a simple associative process following a simple law which seems competent to explain ordinary cases of animal learning. This does not imply that other processes, such as imitation or inference or comparison, may not in some cases mediate between a situation and the animal's reaction to it, that no more complex process than that we have described ever does occur, that this way of profiting by experience is the only way for animals. Decision as to the presence or absence of such processes is not within the province of this paper. Our task so far has been to get a clear idea of what happens in a considerable number of cases of animal learning, and to apply our hypotheses to certain other cases than those from which they were discovered. The task left us is to make some deeper inquiries into the psychology of the process and its possible neural counterpart, to investigate the extent to which such a method of learning prevails down through the animal phylum, the delicacy, complexity, number, and permanence of such associations, and their importance in keeping the animal alive.

We have hitherto remained content with the evident fact that pleasant consequences do stamp in the connection between the impulse and act which led to them and the situation in which the impulse was felt. Biologists in general have been ready to accept this fact. In human life it seems indubitable. Yet it compels one to face a very difficult problem, familiar enough to psychology, but seldom thought out by biologists. For pleasurable feelings of taste, freedom, sexual activity, or what not, are *feelings;* are not facts of motion in space, are not possessed of physical energy, are not amenable to the laws of physical or chemical change. Yet this strengthening of the connection between situation and act means some actual change in the nervous system, some actual physical or chemical readjustment, some actual phenomena of motion in space under the laws of physical and chemical change. If, therefore, one says simply, "The pleasurable feelings are themselves the cause of the neural change which manifests itself in the strengthening of the association," he is saying that a change in the physical world takes place because of the presence of a certain non-physical fact, and has either to admit the transference of physical energy into mental phenomena and back again, or to suppose constant exceptions to the law of the conservation of energy. In the former case we have the marvel of foot-pounds becoming feelings of taste, etc., while in the latter case we have the greater marvel of multitudinous uncaused feelings and multitudinous motions in the nervous system uncaused save by these feelings. A few psychologists, notably Prof. William James, do say so. If, on the other hand, one says, "All consciousness, all feelings are but a fifth wheel, paralleling certain neural facts, but uninfluenced by and not influencing them. The pleasurable feelings do not cause anything. It is their neural counterpart that does it," as most psychologists do say, he finds certain facts hard to reconcile with his hypothesis. The old arguments *pro* and *con* can be found in any elaborate psychological treatise and need not be even mentioned here, especially since the question has been all tangled up with speculations about the freedom of the will, mental activity, voluntary attention, and all sorts of other things. But the question as we meet it in a study of animal behavior is as truly a question of physiological as psychological biology, and certain more or less new and pertinent facts seem worth mentioning. First of all, it is not the impulse and act which are stamped in, but the impulse to that act *in connection with that situation*. No cat goes around clawing, clawing, clawing, because clawing at a button has freed it from a certain box. No child keeps continually chewing because chewing candy has brought pleasure. It is the *connection*, not the act, that is strengthened. Secondly, the pleasurable feeling may not be contemporaneous with the act, but may come considerably later, and the act itself and impulse thereto may not be in the least pleasurable. Our parallelist, therefore, who thinks that the neural counterpart of the pleasurable feeling stamps in the connection, has to show how the nervous activities aroused by nervous currents from the taste buds, due to the presence of fish in the mouth, can find their way around to the cells concerned in the transmission of certain stimuli which came from the retina and went out ten seconds ago to the end plates on the leg muscles, and how, having safely arrived there, they can pick out just the right cells and confirm in them just the right structure, so that when next those currents come from the retina they will be directed in the particular way which shall make the fore leg claw the button. He has, then, further to explain how the neural actions arising from the taste of meat, the feeling of company, sexual activity, etc., all find their way to the same place and all do the same work. If he answers this by saying that the pleasurable element of the feelings is in all cases identical, and that therefore its neural counterpart is identical and acts identically, he has the still harder problem of explaining how any one neural activity can thus set its seal on all sorts of nervous structure anywhere in the nervous system, first convincing us also of the truth of the unlikely proposition that the neural activities corresponding to the taste of fish, sight of other chicks, and sensations from sexual contact have one identical element. A speculative psychologist might attempt all this, but where is the neurologist who would dare?

Yet the problem is not about fine-drawn distinctions, nor a matter for metaphysical speculation, but is a clear question about facts, and facts ever recurring in animal life, and so important in animal economy that they surely ought not to be huddled out of sight. The taste of the meat, or the neural action corresponding thereto, *does somehow* strengthen the association; but for such influence the animal would react the hundredth time as he did the first; by such influence education by experience is possible. The answer, "Feelings of pleasure are themselves true causes," leaves us with a mystery on our hands. The other answer, "The neural counterparts of the pleasurable feelings are the cause," seems to lead straight to neurological monstrosities and myths. I turn over the problem to the neurologists with my blessing.

Apart from this one enigma, the nervous action concerned in the formation of these associations is extremely simple, and is right in line with the nervous action concerned in instinctive performances. Certain stimulation is caused in certain cells by a certain external situation acting on the sense organs. By virtue of inherited organization or acquired habits or accidental influences, this stimulation is reflected into one or two or two-hundred motor impulses. From these one becomes chosen in the mysterious way which we have been discussing. In instinctive activities natural selection has in advance prepared the neural mechanism so that the stimulus is reflected from the start into a limited field, a field which under natural conditions leads to the appropriate act.

I ought, perhaps, to justify my use of the word "association" instead of "habit," especially as Principal C. Lloyd Morgan has used the latter to denote the same resulting phenomena. Neither word is a very good one, for both have in previous usage meant something else than the process I have described. Association has meant the association of ideas, which, we shall see, is a different process in important ways. I try to avoid confusion from this source by calling our process "animal association." Habit has meant the strengthening of the tendency to certain activities by mere repetition, as folds are made in the sleeve of a coat. Now the associations, or habits if you will, which we have been studying are formed in spite of habit in this sense. The cat squeezes at a hole ten times where it claws at the button once, yet the act of squeezing in later trials becomes less frequent, and the act of clawing more frequent. The very essence of the phenomena is that the animal does not *repeat* its acts, but *selects* from among them in utter disregard of the relative number of times each has been performed. The word "habit" can at best describe the result attained, while the word "association" describes both the result and process, and with no more danger of mistake.

It is now high time to consider critically the psychological aspect of these associations, the feelings which the animal has at the time, if he has any. All along I have begged this question, have refused to entertain the supposition that the animal's activities were purely mechanical, automatic, unconscious. I ought to defend this procedure, though probably most of you agree with me that animals do in such cases have consciousness, and have in your own minds good reasons for so thinking. One good reason is that we infer consciousness in the animal for just the same reasons that we do in men, *viz.*, they act as we act when we have thoughts and feelings. Another good reason is that we know of no mechanism capable of changing its reactions to situations, as animals do in the formation of associations, save the human body; and it *does* have pleasurable feelings controlling its activities in just the way we supposed such feelings to do in animals. Without stopping to argue on this question, let me proceed with those who agree that consciousness of some sort is present, to ascertain of just what sort. The word "association" has been used by comparative psychologists in the phrase "association of ideas," and animal behavior of the kind we have been studying has been explained by referring it to the association of ideas, a process which is in a way the A–B–C of human psychology. I see before me a dynamometer, think of Professor Cattell, its inventor; of the words "New York," then of the words "New Amsterdam," then of the Dutch colonists and their multitudinous pantaloons, then of the fitness of all this as an illustration, and end up by the act of taking up my pen and writing. I hear a step, think, "It is the postman," then, "I have a letter to post," and go to get it. These are cases of the association of ideas, and the comparative psychologists would explain our cat's behavior by saying, "When after a number of trials you put the cat in the box, it thinks of the food outside, thinks of going out, thinks of having clawed the button before going out, and so claws at it." I believe that this explanation is totally wrong, that the mental process involved is in no sense the association of ideas of human psychology. It would crowd out more strictly biological subject-matter if I introduced here the data which lead to the rejection of this view, and all these data are accessible to the skeptical in a monograph entitled "Animal Intelligence, an Experimental Study of the Associative Processes in Animals," Supplement No. 8 to the *Psychological Review.* Let it suffice to repeat here the assertion therein justified, that in forming these associations the animals do not think *about* the food, box, or clawing, *at all,* and do not ordinarily connect idea with idea in an associative train of thought; that the association is between a directly felt situation and *an impulse to act.* In the association of ideas, *ideas* are the essential elements; in the animal sort of associations the "*impulse and act*" is the essential. They are homologous, not to our trains of thought, but to our feelings in learning to swim, dive, play golf, etc. They are direct practical associations leading immediately to external acts. From them to associations of ideas is a long step, longer and more important, perhaps, than the step from the latter to logical thinking.

It is hard to answer our next question — "To what extent is this way of learning by the formation of associations prevalent throughout the animal kingdom?" For students of animal life have not had this process clearly in mind and have not collected data with definite reference to it. It will be remembered that illustrations of it in the case of mammals, birds, and reptiles have already been given. Experiments on one of the bony fishes, Fundulus majalis, have demonstrated its presence there. These fish try to get out of the sunlight into a shady corner. If, then, you arrange their aquarium so that one end is shaded, the rest sunny, put them in the sunny end, with some mechanical obstacle between them and the shady end, and watch their actions, you will find that their manner of dealing with the situation in their twentieth trial is different from that in their first, in just the same way that the chick's in the pen or the kitten's in the box was different. They engage in fewer useless acts, perform the successful act much sooner, and so, of course, get back to the shade much more quickly. Let the fish, for example, be in an oblong aquarium ten inches deep. I put a wire screen, which fits across the aquarium, in behind the fish, who is now at the shady end, and move it down toward the sunny end. The fish swims ahead of it, turning often, however, and trying to swim through the meshes of the wire screen back to his habitual haunt. As soon as the fish is at the sunny end, I place across the aquarium, between him and the shady end, a piece of wire screening, impervious save at the upper right-hand corner, where there is a hole 2 × 3 inches (my aquarium was 4 feet long, 2 feet wide, the space in which the fish was now confined being 2 feet long, 10 inches wide, and 10 inches deep), and remove the screen used to push him down to the sunny end. The situation is, of course, "sight of shady end, feeling of the sunlight and of the walls around and screening in front." The animal reacts by remaining still, swimming around, swimming particularly up and down the screening which separates him from the shady end, poking his head against it repeatedly in attempts to swim to the shady end. The swimming is, for the most part, along the bottom of the aquarium, but occasionally the fish rises slowly up and pokes his head against the upper part of the screen. If he does this at the right-hand end, he of course pokes through the hole and gets to the shady end. Sooner or later he is practically sure to do so. If, after giving him fifteen minutes' enjoyment of his preferred habitat, you repeat the experiment, and so again and again, the remaining still and swimming back and forth and poking the head against the bottom of the screening will gradually decrease, until finally the fish, when confronted with the situation, will go directly to the right end, rise up, and glide through the hole without more ado. Moreover, we shall find, when pushing him down with the first screen, that he no longer makes the useless attempts to swim through the screen, which only hurt his head, but swims down to the sunny end as soon as the screen is put behind him. Experiments with different sorts of obstacles give the same results. Evidently the presence of a cerebral cortex is not a prerequisite to the formation of these associations.

How far down in the invertebrate series this process exists remains a question for observation and experiment. Any animal which finds its way to feeding grounds or home apart from direct response to sense-impressions would seem to do so by virtue of associations thus formed.

The Peckhams' study of the solitary wasps convinced them that these insects were gifted with associative powers as well as instincts. If we begin at the extreme end, — with the Protozoa, that is, — we find diverse opinions; but the most recent and by far the most thorough observer, Dr. H. S. Jennings, finds absolutely no signs of any capability to modify reactions in accord with the pleasurable or painful results they bring. At the other end of the scale, in man, associative processes of the animal sort are surely present, though in the adult they are often overgrown and interpenetrated by the ideational life to such an extent as to require, so to speak, careful dissection to make them demonstrable. The human infant, however, learns to cry to obtain certain favors, to go to certain places for certain things, and, up to a certain age, to react to certain words, in just the same way that the animals learn. And in learning certain sorts of novel accomplishments, *e.g.*, to play billiards or tennis, the adult uses to a large extent this same trial-and-error method, progressing because satisfaction stamps in the particular movement or stroke which caused it and makes the player, the next time the same situation appears, more likely to make it. It may be that this is really the basal fact in human intelligence, and that out of it are developed all the higher processes which are, in adult life, so ubiquitous that the ordinary psychological treatise has nothing to say about this simpler sort. Surely the infant, for about nine months, seems to learn in no other way than the animals do. A completer comparative psychology will, let us hope, one day tell us, among other things, when and how this method of learning arose among animals, and how much of a part it has played in the development of the human mind.

Delicacy, Complexity, Number, and Permanence of Associations.

Granted that an animal possesses this associative power, question arises as to the degree of delicacy and complexity which the associations may reach, and as to the number of such associations the individual can acquire and the length of time they last. By delicacy we mean the animal's power of reacting differently to situations varying very slightly; for instance, running toward you when you hold up two fingers and away from you when you hold up three. I may quote what I have said elsewhere on this subject to give an idea of the means of estimating this power in any animal.

Delicacy of Associations.

"It goes without saying that the possible delicacy of associations is conditioned by the delicacy of sense powers. If an animal does n't feel differently at seeing two objects, it cannot associate one with one reaction, the other with another. An equally obvious factor is attention; what is not attended to will not be associated. Beyond this there is no *a priori* reason why an animal should not react differently to things varying only by the most delicate difference, and I am inclined to think an animal could; that any two objects with a difference appreciable by sensation which are also able to win attention may be reacted to differently. Experiments to show this are very tedious, and the practical question is, 'What will the animal naturally tend to do?' The difficulty, as all trainers say, is to get the animal's attention to your signal somehow. Then he will in time surely react differently, if you give him the chance, to a figure 7 on the blackboard from the way he does to a figure 8; to your question, 'How many days are there in a week?' and to your question, 'How many legs have you?' The chimpanzee in London that handed out 3, 4, 5, 6, or 7 straws at command, was not thereby proved of remarkable intelligence or of remarkably delicate associative power. Any reputable animal trainer would be ashamed to exhibit a horse who could not do as much 'counting' as that. The maximum of delicacy in associating exhibited by any animal, to my knowledge, is displayed in the performance of the dog 'Dodgerfield,' exhibited by a Mr. Davis, who brings from four cards, numbered 1, 2, 3, and 4, whichever one his master shall *think of*. That is, you write out an arbitrary list, *e.g.*, 4, 2, 1, 3, 3, 2, 2, 1, 4, 2, etc., and hand it to Mr. Davis, who looks at the list, thinks of the first number, says, 'Attention, Dodger!' and then, 'Bring it.'

This the dog does, and so on through the list. Mr. Davis makes no signals which any one sitting even right beside or in front of him can detect. Thus the dog exceeds the human observers in delicacy, and associates, each with a separate act, four attitudes of his master which to human observers seem all alike. Mr. Davis says he thinks the dog is a mind-reader. I think it quite possible that whatever signs the dog goes by are given unconsciously, and consist only of some very delicate general differences in facial expression or the manner of saying the words 'Bring it,' or slight sounds made by Mr. Davis in thinking to himself the words one or two or three or four. Mr. Davis keeps his eyes shut and his hands behind a newspaper. The dog looks directly at his face.

"To such a height possible delicacy may attain, but possible delicacy is quite another thing from actual untrained and unstimulated delicacy. The difference in reaction has to be brought about by associating with pleasure the reaction to the different sense-impression when it itself differs, and associating with pain tendencies to confuse the reactions.[1] The animal does not naturally, as a function of sense powers, discriminate at all delicately. Thus the cat that climbed up the wire netting when I said, 'I must feed those cats!' did not have a delicate association of just that act with just those words. For it would react just as vigorously to the words, 'To-morrow is Tuesday,' or, 'My name is Thorndike.' The reaction naturally was to a very vague stimulus. Taking a cat just beginning to learn to climb up at the signal, 'I must feed those cats!' I started in to improve the delicacy by opposing to this formula the formula, 'I will not feed them,' after saying which, I kept my word. That is, I gave sometimes the former signal and fed the cat, sometimes the latter, and did not. The object was to see how long the cat would be in learning always to go up when I gave the first, never to do so when I gave the second signal. I said the words in both cases as I naturally would do, so that there was a difference in emphasis and tone as well as in the mere nature of the syllables. The

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[1] It is interesting to know that Mr. Davis says that fourteen months' time, four hours a day, was expended on Dodgerfield's training.

two signals were given in all sorts of combinations, so that there was no regularity in the recurrence of either which might aid the animal. The cat at first did not always climb up at the first signal, and often *did* climb up at the wrong one. The change from this condition to one of perfect discrimination is shown in the accompanying curves, one showing the decrease in *failures* to respond to the right signal, the other showing the decrease in responses to the wrong signal. The first curve is formed by a line joining the tops of perpendiculars erected at intervals of 1 mm. along the abscissa. The height of a perpendicular represents the number of times the cat failed to respond to the food signal in 20 trials, a height of 1 mm. being the representative of one failure. Thus, the entire curve stands for 280 trials, there being no failures after 60 trials, and only 1 after the 40th.

FIG. 4.

"In the other curve, also, each 1 mm. along the abscissa stands for 20 trials, and the perpendiculars whose tops the curve unites represent the number of times the cat in each 20 *did* climb up at the signal which meant no food. It will be seen that 380 experiences were necessary before the animal learned that the second signal was different from the first. The experiment shows beautifully the animal method of acquisition. If at any stage the animal could have isolated the two ideas of the two sense-impressions, and felt them together in comparison, this long and tedious process would have been unnecessary."[1]

By a complex association I mean an association where either situation or act (or both) is a compound. A man can learn that to open a door he has to put the key in the hole, turn it, turn the knob, and pull the door. Or, conversely, a man can learn that when the sound of a bell is followed by a whistle, and that by a blue light, a certain act is in place, while if any one of the three comes alone it is not. In the first case, the *act* is a complex of several; in the second case, the *situation.*

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[1] Animal Intelligence, an Experimental Study of the Associative Processes in Animals, pp. 87-90.

Between such complexity and the *serial* complexity of the association formed by the chick in the labyrinth there is a vast difference. In the latter case situation *A* causes act *B*, which brings the animal into situation *C*, which causes act *D*, etc. This is really a matter of a *number* of simple associations.

Associations that involve complexity in the first sense are very hard for animals to form. If, for instance, you arrange a box so that a cat or dog to escape has to step on a platform, push down a bar, and claw down a string before the door will open, you find that even if the animal is familiar with each of these acts by itself, he will be slow to form the habit of doing the three in combination; will even, after fifty or more trials, not have the association perfect; will frequently step on the platform or claw the string again and again, and will not perform the several acts in any fixed order. The animal does not feel "doing this, doing that, doing the other, getting out," but simply feels, more or less confusedly, intermittently, and in various orders, the three impulses needed.

As regards the number of associations which animals are able to form, I may best quote, with trifling changes, a paragraph or two from the monograph already mentioned:

"The patent and important fact is that there are so few in animals compared to the human stock. Even after taking into account the various acts associated with various smells, and exaggerating the possibility of getting an equipment of associations in this field which man lacks, one must recognize how far below man any animal is in respect to mere quantity of associations. The associations with words alone of an average American child of ten years far outnumber those of any dog. A good billiard player probably has more associations in connection with this single pastime than a dog with its whole life's business. In the associations which are homologous with those of animals man outdoes them and adds an infinity of associations of a different sort. . . .

"Small as it is, however, the number of associations which an animal may acquire is probably much larger than popularly supposed.

"My cats and dogs did not mix up their acts with the wrong sense-impressions. The chicks that learned the series of twenty-three associations did not find it a task beyond their powers to retain them. Several three-days-old chicks, which I caused to learn ten simple associations in the same day, kept the things apart and on the next morning went through each act at the proper stimulus. In the hands of animal trainers some animals get a large number of associations perfectly in hand. The horse Mascot is claimed to know the meaning of fifteen hundred signals! He certainly knows a great many, and such as are naturally difficult of acquisition. It would be an enlightening investigation if some one could find out just how many associations a cat or dog could form if he were carefully and constantly given an opportunity. The result would probably show that the number was limited only by the amount of motive available and the time taken to acquire each. For there is probably nothing in their brain structure which limits the number of connections that can be formed, or would cause such connections, as they grew numerous, to become confused.

"In their anxiety to credit animals with human powers, the psychologists have disregarded or belittled, perhaps, the possibilities of the strictly animal sort of association. They would think it more wonderful that a horse should respond differently to a lot of different numbers on the blackboard than that he should infer a consequence from premises. But if it be made a direct question of pleasure or pain to an animal, he can associate any number of acts with different stimuli. Only he does not form any association until he has to, until the direct benefit is apparent, and, for his ordinary life, comparatively few are needed." [1]

The very fact of the formation of associations is evidence that the connection between situation and impulse and act is permanent. If the influence of the first few successes did not remain to work on the next, there would be no association formed at all. But such permanence is even more clearly witnessed by the behavior of animals who are put in situations with which they have in times past associated certain acts, but

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[1] Animal Intelligence, an Experimental Study of the Associative Processes in Animals, pp. 93–95.

which they have not encountered for a long while. Dogs, cats, chicks, and fishes tested after an interval of from six to eighty days, either manifested as perfect associations as ever, or formed such after far fewer experiences than were needed originally.[1] For instance, the cat that had learned to discriminate between "I must feed those cats" and "I will not feed them" was, after eighty days, given twenty-five trials with the first signal and fifty with the other (all being mingled together indiscriminately). She reacted correctly to every one of the twenty-five signals and by the end of the fifty reacted to that signal as well as she did after 350 trials originally.

The degree of permanence of these associations, once they are formed, vastly increases their utility. It allows experiences rarely met with to still, little by little, build up habits. It allows the experience of certain localities, for instance, to be useful again, even if the locality is not revisited for a considerable time. It lets the animal's past influence its present conduct in all sorts of ways.

It seems hardly necessary to make any statement about the general usefulness of the power of association in securing survival. If our view of the process is correct, it is a process of selection among reactions, not by eliminating the animal that does not react suitably and so developing a stock with certain instincts, but by eliminating the unsuitable reaction directly by discomfort, and also by positively selecting the suitable one by pleasure, and so developing certain associations in the individual. It is, then, selection *within the individual* that is the great case of plasticity, and is of tremendous usefulness, in that it definitely enables the animal to modify his acts and so meet varieties and modifications of environment. New feeding grounds, new foods, new friends, new enemies are dealt with by virtue of it. "He who learns and runs away, *will live* to learn another day."

Teachers' College, Columbia University.

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[1] Only one case out of over forty was an exception to this rule.

SEVENTH LECTURE.

THE BEHAVIOR OF UNICELLULAR ORGANISMS.

HERBERT S. JENNINGS.

In recent biological writings there is manifest a growing tendency to interpret the processes taking place within the bodies of higher animals — especially the developmental processes — as a series of responses to stimuli. In the egg and the developing embryo, masses of protoplasm migrate from one position to another, cells and cell masses alter in form, changes of the most varied character are continually occurring. To explain such changes it is becoming usual to call upon chemotaxis, geotaxis, phototaxis, thigmotaxis, and other motor reactions of similar character. The prevalent ideas of these reactions, known usually under names terminating in *-taxis* or *-tropism*, have been derived to a large extent from the phenomena shown by the movements of unicellular organisms; the classic experiments of Pfeffer on the chemotaxis of bacteria and flagellates and of Strasburger on the phototaxis of swarm spores having opened a fountain from which all have felt entitled to draw. To understand the migration of a cell or mass of cells in the embryo we are referred back to experiments on unicellular organisms, wherein it is shown that the movements of the latter are controlled by chemical agents, by heat, by light, and the like. Here the vital processes are seemingly brought into the closest relation with chemical and physical ones; chemotaxis, for example, is frequently interpreted as the direct expression of chemical affinity or chemical repulsion between the substance of the protoplasmic mass and some other substance, or between two protoplasmic masses. There is thus established an immediate direct relation between the movements of organisms and movements characteristic of inorganic substances; a long step is taken toward that analysis of vital processes into simple chemical and physical ones, which is deemed by many the final goal of biological science. If these phenomena do indeed establish such a relation, they challenge the attention of every man interested in the fundamental phenomena of life; in any case, they invite complete and thorough investigation of the claims made for them. Since it is largely from the reactions of free unicellular organisms that our ideas of chemotaxis, phototaxis, and the like have been derived, it is important to study carefully the reactions of these creatures and to determine the laws which control them. We shall then be in a position to decide whether the movements of these organisms do furnish a key to the understanding of ontogenetic processes or not. It is these considerations that have impelled the investigation whose main results I shall try to present.

In studying the behavior of single-celled creatures we are forced into relation with the much debated question of the nature and importance of the activities of unicellular organisms as compared with those of higher animals and plants. Some hold that the cellular standpoint is the fruitful one for general physiology; that we must first determine the laws of action for single cells, then carry these over to the cell state, understanding the latter only as a combination of the former. Some go so far as to maintain that the reactions of unicellular organisms are of an intrinsically different character from those of higher forms, being of essentially the same nature as the reactions of inorganic bodies; this is, for example, the position of Le Dantec.[1] Others hold that the division of organisms into cells is, physiologically at least, a secondary matter; that nothing more fundamental is to be expected from the study of a unicellular organism than from that of one composed of many cells. This question can be decided, of course, only by a thorough study of both the classes of organisms thus contrasted, with a comparison of the results, to see if the study of the simpler organisms does, as a matter of fact, clear up

[1] La matière vivante, Chapters I and II.

and simplify the phenomena exhibited by the many-celled creatures. For one desirous only of getting at the real laws underlying the phenomena, the conflict on such points between high authorities[1] is very confusing, and the only recourse is to a first-hand study of the facts.

In the hope of getting light on the problems proposed and others of similar character, I shall set forth and discuss observations and experiments made upon a number of free-swimming unicellular organisms. In the investigation it was found well to begin with some single species and work out its activities, and the laws governing the same, completely enough to reveal their essential nature, then to make a comparative study of the activities of other organisms in the light of the knowledge so gained. The same method will be advantageous for the presentation of results.

I give first, therefore, some of the results of a preliminary study of the activities of *Paramecium caudatum*. This is one of the commonest of the ciliate Infusoria, living by thousands in vegetable matter decaying in water. It is a somewhat cigar-shaped creature, having a broad groove passing obliquely from one end (the anterior) to the mouth, which lies at about the middle of the length of the body. The side on which the mouth and groove lie may be called the *oral* side; the opposite one the *aboral* side. The entire surface of the animal is covered with cilia, by means of which Paramecium moves.

In beginning a study of the activities of such an organism, we are at once confronted with the question of its psychic powers. If these unicellular organisms do, as a matter of fact, possess so complicated and highly developed a psychic life as Binet, in his book on the *Psychic Life of Micro-Organisms*, has attempted to show obtains among them, then indeed there is little prospect of gaining light on simple migrations of protoplasmic masses during development, through a study of their behavior. A study of the chick or the dog would perhaps be as promising. The activities which Paramecium shows are at

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[1] See, for example, Verworn, "General Physiology," and Loeb, "Einige Bemerkungen über den Begriff, die Geschichte und Literatur der allgemeinen Physiologie," *Pflüger's Archiv*, Bd. lxix, p. 249.

first view of great complexity, so that they might seem to entirely justify Binet's views as to the height and variety of the psychic powers of these organisms. These activities and their explanation have been discussed somewhat fully by the writer in a paper[1] devoted entirely to the psychological aspect of the matter, so that only so much of this aspect will be taken up at present as has a necessary relation to the questions proposed.

If we place a number of Paramecia, in the culture water in which they are found, upon a glass slide, and cover with the cover glass, we soon find that the animals, which were at first scattered uniformly, have gathered into groups in one or more parts of the preparation. Usually we find that a bit of bacterial zoöglœa forms the center of such a group; as many of the Paramecia as can do so have pressed their anterior ends against the mass, the ciliary current carrying bacteria to their mouths; others press in from behind. It is well known, of course, that Paramecia make no choice in the food which the current brings to their mouths, taking in particles of all sorts indiscriminately. The possibility may suggest itself, however, that they have gathered about these masses of zoöglœa because the latter serve them as food. The choice of food would thus occur a step sooner — the Paramecia choosing their food by gathering about it, then taking whatever comes. But if we introduce into the slide a bit of filter paper or a fine raveling of cloth, we find that the Paramecia gather about it with the same apparent avidity as about the zoöglœa, pressing the anterior end against it and remaining thus, quiet, for long periods.

This and other experiments show, therefore, that this gathering about a bit of bacterial zoöglœa or other substance is not the expression of a choice of food, but is merely a manifestation of the fact that the Paramecia react to contact with solids of a certain physical texture by suspending active locomotion and remaining against the solid. A similar reaction to solids is, of course, a very common phenomenon among organisms of different sorts; it has received the name "Thigmotaxis," or "Stereotropism."

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[1] "The Psychology of a Protozoan," *Amer. Journ. of Psychology*, vol. x, No. 4, 1899.

We have in thigmotaxis one of the fundamental reactions of Paramecium, not further analyzable into simpler component reactions. As it seems to consist chiefly or entirely of a cessation of a part of the usual ciliary motion, — only the cilia in the oral groove continuing to strike strongly backward, — it may be more philosophical to consider this partly resting condition as the "normal" condition, the usual forward motion being then considered a reaction to a stimulus, due to a change or removal of the solid body against which the animal is resting, or to some other change in the environment. There seems to be no decisive reason for considering either the condition of partial rest or of the usual forward motion as more "normal" than the alternative condition; taking either as a starting point, the other may be considered a response to a stimulus.

If the Paramecia are placed upon the slide in pure water, containing no bacterial zoöglœa, or any other solid, they do not even then remain scattered uniformly throughout the preparation. On the contrary, it is usually not long before the animals are gathered into one or more close groups in some part of the slide. Paramecia are usually found in the culture jars also aggregated into groups; this, taken together with the above experimental demonstration that Paramecia, at first uniformly scattered, will soon collect into close groups without evident external cause, might be held to indicate the existence of a "social instinct" among these creatures. Another possibility suggests itself — that there may be some invisible chemical substance in the region of these groups by which all the Paramecia are attracted; so that the fact that they come near together would be a secondary result of the fact that all are attracted by the same substance.

The main results of the extended study of the conduct of the Paramecia toward chemicals, to which this possibility led, may be given in a few words. It was found that Paramecia tend to gather together and form collections in drops of weakly acid solutions, and in solutions of some salts, while they avoid alkaline solutions and solutions of the salts of the alkali metals.

Among the substances into solutions of which they gather is carbon dioxide. If a bubble of carbon dioxide is introduced into a preparation of Paramecia, they soon collect closely about it and swim in circles around it without leaving it.

It was then proved, by introducing the Paramecia into a solution of rosol, which is decolorized by carbon dioxide, that these Infusoria excrete a distinctly appreciable amount of this substance, which diffuses into the surrounding water. Whenever, therefore, a very few Paramecia get together, an active solution of carbon dioxide is soon formed, and the region becomes at once a center of attraction for the Paramecia. A most complete correspondence was demonstrated between the diffusion of the CO_2 into the water and the distribution of the Paramecia in groups, and all the phenomena exhibited by the (apparently) spontaneous collections of Paramecia could be exactly imitated by introducing CO_2 into the slide.

Thus these collections of Paramecia give no indication of "social instinct," but are merely the expression of positive chemotaxis on the part of the animals toward a certain substance. In the same way all the seemingly complex activities of these creatures may be reduced to simple factors, so that there seems no evidence to indicate the possession by them of psychic powers of anything more than the most elementary character.

We may proceed then to a closer analysis of the apparent attractions and repulsions — chemotaxis, thermotaxis, and the like; it is from a study of these that light is to be gained on the problems first proposed. We shall first consider chemotaxis.

The fact that animals and plants are attracted by certain chemical substances and repelled by others is of course well known for a large number of organisms. As to the essential nature of this phenomenon, opinions differ. As pointed out above, some hold that chemotaxis is the direct expression of chemical affinity or repulsion between the living protoplasm and the chemical. Le Dantec (*La matière vivante*, pp. 51, 52) gives geometrical figures illustrating the action between the surface of a free cell and a chemical substance diffusing in the surrounding water, demonstrating in mathematical form that as a result of this action the cell must move either toward or away from the center of diffusion of the chemical. The motion of such a protoplasmic body would be passive in the same sense as the movements of iron filings are passive when acted upon by a magnet. Delage and Herouard[1] actually state that the Flagellata have, in addition to their usual active movements, also a *passive* motion, due to the attraction of chemical substances. Perhaps the majority of biologists hold less radical views than this; yet the opinion seems widespread that in chemotaxis we are dealing with a simple primary phenomenon.

Coming now to an examination of the phenomena as exhibited by Paramecium, we will first take up positive chemotaxis, or attraction toward chemical substances. The phenomenon to be explained shows itself as follows. If into a slide of Paramecia a drop of some attractive substance (as a weak acid) is introduced, the Infusoria soon collect in the drop, forming there a dense assemblage. Now, what is the exact action of the attractive substance on the Paramecia to cause them to turn and enter the drop? Observing carefully the conduct of the animals, we find, first, that *they do not* turn toward the drop. Owing to its slow diffusion, the margin of a drop thus introduced beneath the cover glass is evident, and the Paramecia, swimming in every direction throughout the preparation, may be seen in their random course to graze almost the edge of the drop, without their motion being changed in the least; they keep on straight past the drop and swim to another part of the slide. But of course some of the Paramecia in their random swimming come directly against the edge of the drop. These do not change their motion, but keep on undisturbed across the drop. But when they come to the opposite margin, where they would if unchecked pass out again into the surrounding medium, a marked reaction is caused; the Paramecium jerks back and turns again into the drop. Such an animal then swims across the drop in the new direction till it again comes to the margin, when it reacts negatively, as before. This continues, so that the animal appears as if caught in the drop as in a trap. Other Paramecia enter the drop in the same way and are imprisoned like the first, so that in time the drop swarms with the animals. As a result of their swift, random movements when

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[1] Traité de zoologie concrète, tome i, p. 305.

first brought upon the slide, almost every individual in the preparation will in a short time have come by chance against the edge of the drop, will have entered and remained, so that soon all the Paramecia in the preparation are in the drop.

Thus it appears that the animals are not attracted by the fluid in the drop; they enter it by chance, without reaction, then are repelled by the surrounding fluid. The peculiar fact that the animals, after entering the drop of the substance in question, are repelled by the surrounding fluid in which they were previously immersed will become more comprehensible after the phenomena of repulsion are considered.

Turning, then, to the matter of negative chemotaxis or repulsion, we have the following phenomenon to be explained. If into a slide of Paramecia swimming at random a drop of some repellent chemical (as NaCl) is introduced, we find that the drop remains entirely empty, not a single Paramecium entering it. Now, exactly how do the Paramecia succeed in keeping out of such a repellent solution?

Careful observation shows that when the Paramecium, swimming forward, comes in contact with the drop of repellent substance, it swims backward a short distance, then turns *toward its own aboral* side, then swims forward again. The essential point in this reaction method is, that the Paramecia always turn toward their own aboral side, without regard to the position of the stimulating drop. If a Paramecium comes obliquely in contact with the drop so as to touch it only on one side of its body, it nevertheless gives the reaction above described without modification, even though turning toward its own aboral side after backing off may carry the animal directly toward the drop, instead of away from it. In such a case the animal when it comes again in contact with the drop simply repeats the reaction. As it continually revolves on its long axis both when swimming forward and when swimming backward, the aboral side is nearly certain to lie in a new position the second time, so that the animal turns in a new direction. If this is repeated a sufficient number of times, the Paramecium is fairly certain, by the laws of chance, to get started finally in a direction which carries it away from the stimulating chemical.

It thus appears that the direction in which a Paramecium turns after stimulation by a chemical substance is not determined by the position of the stimulating agent, nor indeed by any external factor, but by an internal factor, — by structural differentiations of the animal's body. This is demonstrated in a striking manner by immersing the Paramecia directly into a chemical solution of such a nature as to act as a stimulus. The entire surface of the animal is then bathed by the chemical, so that there is nothing in the external conditions to determine in which direction the animal shall move. Nevertheless, under these circumstances, it swims backward, turns toward the aboral side, and swims forward, usually repeating the operation indefinitely. Very striking is also the experiment of causing the chemical to act first upon the posterior end of the animals. This may be done as follows: A large number of Paramecia are frequently observed with anterior ends pressed against the surface of a bit of bacterial zoöglœa (thigmotactic reaction), so that the posterior ends are all pointed in the same direction. Now, a capillary glass rod, coated with some chemical, is introduced into the water behind the Paramecia. The chemical gradually diffuses through the water, of course first reaching the posterior ends of the Paramecia. But these, when they respond, react exactly as in the other cases; they swim backward some distance and turn toward the aboral side. It often occurs that in thus swimming backward they enter the densest part of the chemical and are killed by it.

These experiments indicate that not only the direction of turning after swimming backward, but also the swimming backward itself is determined by internal factors, and is independent of the position of the source of stimulus. This conclusion seems strictly true for chemical stimuli both in Paramecium and in other Infusoria experimented with. As will be shown later, other experiments throw a light upon the cause of this uniform backward motion when stimulated by a chemical.

Summing up, then, we may say that when Paramecium is chemically stimulated it swims backward, turns toward its own aboral side, then swims forward. As a rule, the anterior end, moving forward, comes first in contact with the chemical, so that swimming backward does, as a matter of fact, usually carry the animal away from the source of diffusion of the chemical, and turning toward the aboral side before swimming forward again will, as a rule, if repeated, finally carry the animal in such a direction that it does not again come against the source of stimulus. But these are, from the physiological standpoint, matters of accident; the animal conducts itself in the same way whether the source of stimulus has this usual position at the anterior end of the animal or not. The direction of motion after a chemical stimulus, then, has no relation to the position of the chemical substance. We cannot say, therefore, that the Paramecia are *repelled* by any chemical substance — just as we were compelled to conclude that they are not directly attracted by any chemical substance.

We find, then, that the effect of chemicals on Paramecia is not to attract or repel them, but simply to cause a certain set formula of movements. Such a set formula of movements, "touched off," as it were, by stimuli of various sorts, may be called a reflex. In returning now to the question of how the apparent attraction of the Paramecia toward certain substances — that is, the fact that they collect in drops of certain substances — can be brought about through such a reflex, it is necessary to recall certain general facts in regard to the nature of reflexes. First, any change in the environment that can be perceived by the organism may "touch off" such a reflex. Second, the character of the reflex has no necessary relation to the nature of this external change, so that of a given kind of change it cannot be predicted beforehand whether it will cause the reflex or not, and changes of opposite character may produce the same reflex.

The mechanism of the gathering together of the Paramecia into a drop of some weak acid is then as follows: When the Paramecium passes from the surrounding fluid into the acid solution there is, of course, at the moment of crossing the boundary of the drop a change in its environment. Whether this change will cause the characteristic reflex or not is impossible to predict, that depending upon the internal mechanism of the organism; as a matter of fact we find that it does *not* cause the reflex. Now, after passing across the drop it comes again to the boundary where, if not stopped, it would pass out again into the surrounding fluid. At this boundary there is, of course, another change in the environment — a change in the opposite sense from that experienced in passing into the drop. Whether this second change will cause the reflex is of course likewise impossible to predict, since it depends upon the nature of the organism ; as a matter of fact we find that it *does* cause the reflex. The Paramecium is, therefore, returned into the drop and kept there in the manner already described. It seems probable that the physiological condition of the Paramecium is changed by immersion in the drop of acid, so that contact with the culture fluid now acts as a stimulus, though it before did not. It seems not impossible to conceive, however, that even without such a change in physiological condition, an environmental change from *b* to *a* might cause a reaction, when the opposite change, from *a* to *b*, would cause none. This has, as is evident from the nature of a reflex, no necessary relation to the comparative actual mechanical difficulty in passing in one direction or the other.

The one effect of a marked chemical stimulus on Paramecium is, then, to produce the characteristic reflex already described, and the apparent attraction or repulsion is determined by the fact that some chemical substances or chemical changes cause the reaction, while others do not.

Now, experimentation with stimuli other than chemical leads to the highly important observation that this same reflex is produced by stimuli of the most varied nature. Substances which seem to act upon Paramecium only through their osmotic pressure, such as solutions of sugar, cause the same reflex ; tonotaxis, then (to use the name employed by Massart), acts through the same reflex as does chemotaxis. Mechanical stimuli, produced by jarring the preparation, cause the same reflex. Heat and cold act in the same way, and the Paramecia avoid hot or cold areas and collect in regions of optimum temperature in exactly the same manner as they avoid certain chemicals and collect in others.

We are driven, therefore, to the conclusion that chemotaxis is not an activity differing in kind from the other reactions of these animals. Many sorts of changes in the environment produce a certain characteristic reflex in Paramecia, resulting in their collecting in regions of certain characters and leaving other regions vacant. Among the changes that act thus are chemical changes, and the characteristic groupings of the animals so caused are said to be due to chemotaxis; they are, however, produced in an essentially similar manner to the groupings produced by other agents. There is a unity underlying the motor activities of the Paramecia — a unity expressed in the fact that the different classes of stimuli produce identically the same reaction.

To be accurate, however, we must distinguish two less important forms of reaction to stimuli that are not manifested through the characteristic reflex above described. One is thigmotaxis; this is, however, not a motor reaction, but one characterized chiefly or entirely by a cessation of a part of the usual motion. Again, as previously set forth, it is possible to consider the partially resting condition characteristic of thigmotaxis as the primary condition; then the ordinary forward motion of the animal will be a motor reaction to a stimulus, since it is induced by a change in the environment. As will be shown, there is sufficient ground in certain other Infusoria to *compel* us to consider this forward motion as at times a reaction to stimulus; this, then, is a motor reaction which does not take place through the above-described characteristic reflex. It seems possible that the following represents the true state of the case; very weak stimuli acting on the resting individual cause the ordinary forward motion; stronger stimuli produce the above-described motor reflex.

In view of the means by which chemotaxis is brought about, it becomes more intelligible why the Infusoria may at times collect in regions of injurious substances and avoid at times areas of harmless substances. It is not a matter of attraction or repulsion at all. In the former case the injurious substance merely does not act as a stimulus to cause the motor reflex; in the second case, the chemical in question, though not injurious, does act as a stimulus. An extended investigation directed upon this point showed that the chief factor determining whether a substance does or does not cause the motor reflex of Paramecium is not the injuriousness of the substance, but is of a chemical nature.

We are now prepared to sum up the main results on Paramecium. In this animal we find that chemotaxis, thermotaxis, tonotaxis, reactions to mechanical shock, and the like, are not distinct kinds of activity; that in each case we have the same movements, merely induced by different agents. When Paramecium is effectively stimulated by any substances acting chemically or through osmosis, by heat or by cold or by mechanical shock, it responds with a reflex, which consists of the following activities: the animal swims backward, turns toward its own aboral side, then swims forward. The result of this method of reaction is that the Paramecia tend to leave the sphere of influence of agents causing this reflex, and to congregate in areas where this reaction is not caused. For chemical substances at least it is proved that the position of the stimulating agent has no influence on the direction of movement after a stimulus; the direction of movement throughout the reaction is determined by internal factors.

Is this reaction method one that is common among unicellular organisms, or is it peculiar to Paramecium? To answer this question I have studied the reactions of a considerable number of unicellular organisms belonging to the Flagellata and Ciliata. The essential point in the reaction of Paramecium, the factor that gives character to the entire response, is the circumstance that the animal after stimulation turns toward one side which is structurally defined, without regard to the nature and position of the source of stimulus. The point to which attention was primarily directed in studying the other organisms was, therefore, whether after stimulation the creature turned always toward one structurally defined side.

The organisms studied included, among the flagellates: *Chilomonas paramecium* and *Euglena viridis;* in the ciliates the following Holotricha: *Paramecium caudatum*, *Loxophyllum meleagris*, *Colpidium colpoda*, *Microthorax sulcatus*, *Dileptus anser*, *Loxodes rostrum*, and a species of *Prorodon;* the following Heterotricha: *Stentor polymorphus*, *Spirostomum ambiguum*, and *Bursaria truncatella*; of Hypotricha, *Oxytricha fallax* and a number of undetermined species. In several of these creatures, on account of the large size or other favorable circumstances, it was possible to use methods of investigation not available for Paramecium; in particular it was possible in a number of cases to localize very precisely the action of stimuli.

In all of the organisms named, in spite of great variations in the nature and complexity of the usual movements, the reaction method was essentially similar to that of Paramecium. In all, the direction of turning after a stimulus was toward a structurally defined side, without regard to the nature and position of the source of stimulus. With regard to the details of the reaction, as might be expected, the greatest variety exists, but the general reaction plan was the same throughout.

This method of reaction evidently has a close relation to the usual asymmetry of the cell body exhibited by these organisms. This asymmetry of the Infusoria has also a close relation to the normal method of progression through the water, as well as to the method of reaction to a stimulus. Most of these organisms, as they swim forward, also revolve on the long axis, and the resulting path is usually a spiral. The form of the body has a constant relation to the axis of the spiral, the same side being at all times directed toward this axis. The unsymmetrical structure of the body, the usual method of progression, and the method of reaction to a stimulus are thus evidently closely interrelated. In the case of a bilaterally or radially symmetrical animal one would certainly not expect that one side would be always preferred to the other in turning away from a source of stimulus, as is the case in the Infusoria.

In the case of chemical stimuli it was found for all the organisms studied that not only the turning to one side, but the swimming backward after a stimulus, was independent of the position of the source of diffusion of the chemical. The action of chemical stimuli was localized by bringing a capillary glass rod coated with some chemical compound near the anterior end, one side, or the posterior end, of the quiet organisms. In every case (except in *Euglena viridis*, which does not swim backward under any circumstances) the organisms reacted to the chemical stimulus by swimming backward, turning toward the usual structurally defined side, then swimming forward. The swimming backward, of course, sometimes carried the creature away from the densest part of the solution (when the chemical was held in front); at other times, directly toward and into the densest part (when the same chemical was held behind). In the latter case the organisms were frequently killed by swimming into the dense solution. Thus, in chemical stimuli, without exception, the direction of motion after stimulation has no relation to the localization of the stimulus.

In several of the organisms it was possible to use also very precisely localized mechanical stimuli; and the results so gained tend to modify in some particulars the general conclusions that might be drawn from a study of the action of localized chemical stimuli. Localized mechanical stimuli were produced by touching under a powerful lens any desired part of the body of the organism with a glass rod drawn to the finest hair in a flame. For Paramecium itself this method of experimentation was not satisfactory, owing to the minute size of the cell body. One point of importance was brought out in Paramecium, however. The anterior tip of the body was shown to be incomparably more sensitive than any other part. On bringing the glass hair near the anterior tip, Paramecium leaps backward almost before the hair is seen to have reached it, giving the entire typical reaction already described. Any other part of the body was so insensible that it was not possible to cause a reaction of any sort by touching it with the hair. Paramecium could be pushed about and made to alter its direction of movement mechanically, of course, but there was no active response of any sort when it was touched at any point except the anterior end.

In *Spirostomum ambiguum* essentially the same results were reached with mechanical as with chemical stimuli. If any part of the body was touched, whether anterior end, posterior end, or side, the infusorian gave the typical reaction — swimming backward, turning toward the aboral side, then swimming forward. A slightly greater percentage of cases of the typical reaction was obtained by touching the anterior end, but the difference was little; it varied in Spirostoma from different cultures.

In the other organisms on which the effects of localized mechanical stimuli were tried, particularly *Loxodes rostrum*, *Dileptus anser*, *Oxytricha fallax*, and one or two other Hypotricha, the following results were obtained: (1) The side toward which the animal turns after a stimulus is entirely independent of the side which is touched. In every case the organism turns toward one structurally defined side. If that is the side which is touched, the organism turns continually toward the source of stimulus, no matter how many times the latter is repeated; if the other side is touched, the creature of course turns away from the source of stimulus. The impression is given that it is physiologically impossible for the organism to turn otherwise than toward this one side. (2) But the forward or backward movement of the animals after a stimulus is *not* thus independent of the localization of the stimulus. If the anterior end is touched, the organism darts backward, turns toward one side, then swims forward. The posterior half of the body is very insensible, so that as a rule there is no response to a mechanical stimulus occurring here. If, however, a strong stimulus is given here, as by thrusting the tip of the rod strongly against the resting animal, the latter simply *swims forward;* if already swimming forward, it merely hastens its forward motion when thus stimulated.

Thus, in the case of mechanical stimuli in these organisms the direction of motion after a stimulus depends, *to a certain extent*, so far as backward or forward motion is concerned, upon the localization of the stimulus. This introduces a greater complexity into the psychology of these creatures than the results on Paramecium alone, or on the reactions to chemical stimuli alone would lead us to judge. The organisms do in certain respects react with reference to the localization of a stimulus affecting them. The differing results gained with chemical stimuli are probably to be interpreted, in view of the facts shown by a study of mechanical stimuli, as follows: When a chemical diffuses from a point lying behind the infusorian, it of course comes first in contact, as a very weak solution, with the posterior end of the animal. Now, as already stated, this posterior end is not at all sensitive, so that no reaction is caused. The chemical continues to diffuse until it finally reaches the very sensitive anterior end, when at once the typical reaction occurs, and the animal swims backward into the strong solution. The reaction to a chemical is perhaps then always due to stimulation at the anterior end.

Psychologically considered, we seem to have here a remarkable transitional condition toward a perception of the localization of the stimulus by the organism — a reaction with reference to the localization of the stimulus so far as motion along the axis of the body is concerned, a blind reflex, without regard to the localization of the stimulus, so far as motion to one side is concerned.

We may now summarize briefly the essential facts in regard to the reactions of the unicellular organisms studied. The reactions of these organisms may be classified into three reaction forms:

(1) One is the thigmotactic reaction. Starting with the moving infusorian, we find that it reacts to contact with solid bodies of a certain physical texture by suspending part of the usual ciliary motion, so that locomotion ceases and the organism remains pressed against the solid. Whether anything more than this cessation of part of the usual ciliary motion is concerned in the thigmotactic reaction is very difficult to say.

(2) If we start with the resting individual, the simplest reaction to a stimulus is the resumption of the usual forward motion. This is the reaction that is produced when the solid substance against which the creature is resting is removed; it is also produced in some Infusoria when the posterior part of the body is stimulated mechanically.

(3) The third, and, for our purpose, most important reaction, to which most of the so-called tactic or tropic phenomena are due, may occur in either active or resting animals. It is a reflex consisting of the following activities: the animal swims backward, turns toward one structurally defined side, then swims forward. This reaction is produced by chemical stimuli acting upon any part of the body or upon the entire body at

once, by osmotic stimuli, by heat, by cold, by mechanical shock. Its general effect is to take the organism out of the sphere of operation of the agent causing the stimulus, and to prevent it from reëntering. The fact that certain areas are left vacant is because the agencies within these areas cause this reaction; the collecting of the organisms within certain areas is due to the fact that here the reaction is not produced, while it *is* caused, by the influences active in the surrounding regions.

Thus, chemotaxis, tonotaxis, thermotaxis, and the like are unified; they are not qualitatively different activities, but are fundamentally one activity due to different causes. The tactic phenomena of unicellular organisms are brought throughout under the same point of view as the motor reflexes so well known in the physiology of higher animals.

We may now return to a brief consideration of the problems which formed the starting point of this investigation — the relation of the phenomena studied to the growth processes in the protoplasmic masses of higher organisms. Do the laws of the motor reactions of unicellular organisms, chemotaxis and the like, really give us a basis for the understanding of protoplasmic migrations and other processes in growth and differentiation?

We find that the tactic phenomena of these unicellular forms are brought about through a reflex that is in all essential points similar to the reflexes of higher animals. The nature of this reflex is closely bound up with the physiological and structural differentiations of the body of these organisms; it has a specially close relation to the asymmetry of the cell body in these Protozoa, and to the manner of the usual forward motion. These differentiations are absent in the masses of protoplasmic substance that are moved about in the processes taking place within the eggs and embryos of Metazoa. It is difficult to see how the laws controlling the movements of such substance masses can have any similarity to the laws above developed for the reflexes of free unicellular organisms. Above all, it is evident that the tactic movements of unicellular organisms are not direct expressions of simple chemical and physical laws; chemotaxis, for example, is not a direct result of chemical affinities and repulsions between the protoplasmic substance and other chemical compounds. Like all the other tactic phenomena, it is the result of a motor reflex, which may be produced by the most varied means. These tactic movements, then, do not establish an immediate relation between the movements of organisms and the movements characteristic of inorganic substances. The organism reacts as an individual, not as a substance. To my mind the facts above brought out in regard to the movements of these creatures tend, if these facts have a general validity, to deprive such movements of their supposed value for explaining or illustrating the processes of growth; in so far as the ideas of chemotaxis and the like in growth processes have been derived from the phenomena exhibited by unicellular organisms, these ideas require a revision.

Especially do the facts above brought out reveal the fallacy of the statement so often insisted upon, that the growth processes induced by chemical or physical agencies are "the same as" or "identical with" the locomotor reactions induced by the same agencies. This has been carried so far that strenuous objection has been raised even to the use of distinguishing terms for these two sets of phenomena. We are told that to distinguish as *-taxis* the motor reactions of a free organism from *-tropism* or the growth reactions of a fixed organ or organism is all wrong; the two are "identical." It is reasonably certain that the growth phenomena of plants are not brought about through a reflex that is identical with the motor reflex of Paramecium; it seems exceedingly probable that the ways by which movements are brought about as responses to stimuli in the various classes of plants and animals will present great variety. It is difficult to see what is to be gained except confusion of ideas by applying the same names to two such dissimilar activities as the motor reflex of Paramecium when stimulated by a chemical, and the bending of a plant to or from a chemical in solution.

In regard to the second question touched upon in my introduction, — the nature and importance of the activities of unicellular organisms as compared with those of many-celled creatures, and their value for explaining the phenomena shown by the higher, — the general trend of the answer is, I think, evident. I should be inclined to interpret the facts presented somewhat as follows: The claim that the motor processes of unicellular organisms form a connecting link between inorganic processes and the vital phenomena of higher creatures clearly receives no justification for the organisms studied. Every influence coming in from outside passes, as it were, through a sort of central station, where it is completely transformed to appear as a reflex action, the nature of which is conditioned by the form and structure of the organism; and the steps in the transformation are no more evident than they are in the higher forms. The reactions of these creatures are indeed simple, but not qualitatively of a different sort from those of higher organisms, so that for motor reactions of the sort studied I do not see that a knowledge of the conduct of these particular unicellular organisms really adds to our insight into the causal relations in the activities of higher animals.

On the other hand, if we dismiss any idea of getting from them knowledge of a different kind from that gained by the study of other groups, then the behavior of these Protozoa is of the greatest interest from the standpoint of comparative psychology. In these creatures we see, as nowhere else, how activities that seem so complicated and varied as to require psychological powers of a high order, are produced merely through one or two simple reflexes; it seems not impossible that the phenomena exhibited in the conduct of these organisms may in time furnish important points of support for the general theory of the origin and development of psychic powers.

EIGHTH LECTURE.

THE BLIND-FISHES.

CARL H. EIGENMANN.

> "An investigation into the history of degenerate forms often teaches us more of the causes of change in organic nature than can be learned by the study of the progressive ones." — WEISMANN.

THE Amblyopsidæ are a small family of fishes of six or seven species. Four or five of these live in the caves of the Mississippi Valley. Amblyopsis is the largest of the species and has the widest range, being found both north and south of the Ohio River, and reaching a length of 135 mm. The structure of these fishes will be dealt with in various journals. The habits in general are given in the *Popular Science Monthly* for 1900. I shall consider here the reaction to light and the breeding habits of Amblyopsis and the color of the Amblyopsidæ.

I. *Reaction to Light.*

A long series of observations and experiments were made to determine the reaction of Chologaster and Amblyopsis to white and monochromatic light. Incidentally other characteristics were brought out. The experiments need not be given in detail here.

Some previous experiments on blind or blinded vertebrates may be recalled. Dubois[1] and Semper[2] record that Proteus, the blind salamander of Europe, is sensitive to diffuse light. Graber[3] records that blinded salamanders prefer dark chambers to light ones. Korange[4] notes that concentrated light deprived

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[1] *Compt. Rend.*, 110, pp. 358-360.

[2] Animal Life, p. 79.

[3] *Sb. Akad. Wiss.*, 87, pp. 221.

[4] *Centralbl. of Phys.*, 6, pp. 3-6.

of heat rays, thrown upon the leg of a frog whose brain had been laid bare and covered with extract of beef caused it to respond each time with reflex movements.

Amblyopsis seeks the dark, regardless of the direction of the light. An aquarium about eight feet long was placed in the open, with the darkened end toward the north. The fishes remained in the dark area all day, coming out at night. A smaller aquarium was divided into two compartments, at first with but a small connecting opening; later with a double partition, with an opening at one end of one partition and another opening at the opposite end of the second partition. One compartment and the connecting tunnel were dark. At first there was a constant change of individuals from the dark to the light chamber, but after several days they remained in the dark chamber during the day. These experiments were conducted in my laboratory where usually only diffuse daylight prevailed. Jarring the aquarium or changing the water was usually sufficient to cause them to come out into the lighted compartment. It may be stated that the fishes found no difficulty in finding the opening connecting the compartments, and that the fishes in the dark were continually swimming past the opening without attempting to come out. Rarely one passed out into the light chamber, and then invariably showed signs of uneasiness, frequently turning sharply and reëntering the dark chamber.

Four blind-fishes which had been kept for a day in a vessel painted black, and covered to exclude the light, were experimented upon as follows: a ray of light from a mirror about two inches in diameter was thrown on each successively. After from one to five seconds the fishes became uneasy, the uneasiness giving place to discomfort, finally resulting in vigorous effort to get out of the way.

Another jar, not painted, containing both blind-fishes and blind Cambarus, was placed where light could be reflected upon them from the mirror of a microscope. The Cambarus, if in motion, came suddenly to a halt; if quiet, backed or moved off at once. The fishes also responded to the reflected light, but it took several times as long for them to do so.

Bright sunlight appears to be irritating; if exposed to it, the fishes swim about uneasily. A shadow passed suddenly across them, when in the diffuse light of a room, does not affect them; nor did they seem disturbed, when swimming through a ray of light entering the dark chamber by a small hole in the paint made for the experiment.

Two examples kept in a pail in my cellar were quietly floating, but when a lighted match was held above them, the fishes at once darted to the bottom and sides of the pail. The heat, in this case, could not have been a factor; the reaction to the light of the match was quick and violent. The same observation was made on forty individuals in two aquaria. They were captured one morning, and the observation made the second night after. They had been kept in the dark during most of the intervening time. A lighted match, held near the aquaria, produced a very general and active movement among all the individuals.

A series of observations was made to determine to what rays, if any, they reacted most vigorously. Only the concluding data need be given here.

For this experiment a glass jar, three feet long and eight inches in diameter, was divided into six compartments by five partitions. Each partition had a vertical slit extending halfway up from the bottom to enable the fish to swim freely from one compartment to another. The compartments were thus all connected. A cap was screwed tightly over the end of the jar, which was placed horizontally on a window sill where each compartment would have an equal amount of light. The jar was surrounded with bands of tissue paper in several layers of violet, blue, green, orange, and pink, so that each compartment was lighted by one series of rays.

Three blind-fishes were used for these observations; they were selected for their size, and numbered: 1, the smallest; 2, the middle-sized; 3, the largest. These fish had been in confinement some time, but had been transferred from the cave, with as little exposure to light as possible, to a dark room, where they were very seldom exposed to the light.

It was found that certain compartments were visited by a certain fish without any definite regard for color. During January, for instance, fish 3 moved out of the pink and orange compartments but once; fish 1 remained almost exclusively in yellow, visiting pink once, orange once, and green four times. Fish 2, on the other hand, remained mostly in violet, visiting blue seven times and green three times. From this we must conclude either that different individuals react differently, or that one color does not produce a stronger reaction than another, and the latter seems the more reasonable conclusion.

To determine whether the apparatus had anything to do with the distribution, and also whether widely separated elements of the spectrum would cause the fish to react positively or negatively, they were put into a rectangular aquarium impervious to light, except at the ends, and divided by a median partition. The ends were covered with translucent celluloid film, care being taken, of course, to have each end equally light.

In these experiments there was again conflicting evidence. In general, it may be said that there was no marked difference in the reaction to one or another of the rays of the spectrum.

A similar series of experiments was conducted with Chologaster with the same reaction to white light, but with a marked positive attraction by the red rays of the spectrum as against the blue.

A very positive series of observations has been made since this was written. An aquarium two feet deep, four feet wide, and about eight feet long was divided by a median partition to near the bottom. Two lids were hinged at the top of this partition. One or the other or both sides of the aquarium could be covered at pleasure. Invariably, within a reasonable time after one of the compartments was uncovered, all the fish migrated to the dark compartment.

Even more striking than this is the action of a colony of Amblyopsis in an open pool. During the bright part of the day, the fishes always remained under the rocks at the bottom. In the morning and evening and at night, they could be seen swimming about in various parts of the pool.

II. *Breeding Habits.*

We owe the first observations on the breeding habits of Amblyopsis to Thompson,[1] who states that a fish "was put in water as soon as captured, where it gave birth to nearly twenty young, which swam about for some time, but soon died. . . . They were each four lines in length." Little or nothing has been added to our knowledge of this subject since that time; but the highly interesting supposition of Thompson, that they were viviparous, has gained common currency, and it is therefore unfortunate that in this respect he was in error.

Putnam[2] adds to the above that, judging from some data in his possession, the young are born in September and October, and further along remarks that they are undoubtedly viviparous.

The first young I obtained were secured on May 9, 1896. The little fish could move actively for a few moments, but as they were encumbered with much yolk, they soon settled to the bottom and remained quiet. There were a large number of old ones in the water in which the young were found, and the mother of this lot was not identified with certainty. Another lot of young were obtained on September 5 of the same year. These were much further along in their development than the first lot secured. Some were preserved and others were placed in various aquaria, where one lived to be ten months old. As before, the parent was not with certainty determined, simply because it was taken for granted that they were viviparous, and the ovaries only were examined. Two other lots of young were obtained on June 5, 1897. One of these lots was in the stage of the first lot obtained, with a large amount of yolk still present, while in the other lot the yolk had almost entirely disappeared. These had been carried in the gill cavity of the mother, and it became evident that either the fish were not viviparous at all, or their viviparity was not nearly of the pronounced character hitherto supposed.

On March 11, 1898, twenty-nine individuals were captured. Four of these were females with eggs in their gill cavities.

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[1] *Ann. Mag. Nat. Hist.* 1844.

[2] *Am. Nat.*, p. 16, 1872.

The youngest stage among these was at the end of segmentation, the oldest was a gastrula covering but one-third of the yolk. The eggs had not been developing more than five days, probably not more than two at the utmost, and decided beyond a doubt that the fish are oviparous, and not viviparous.[1]

The eggs are laid by the female in under her gill membrane. Here they remain for perhaps two months, till the yolk is nearly all absorbed. If at any time a female with young in her gill pouches is handled, some of them are sure to escape. This was observed, and gave rise to the idea that this fish is viviparous. Eggs have been obtained as early as March 11 and as late as September, and the indications are that the breeding season extends throughout the year.

The eggs are translucent amber, in various shades in different individuals. They are large, measuring 2.3 mm. in diameter from membrane to membrane. The yolk measures 2 mm. in diameter and contains an oil globule 1.2 mm. in diameter. The globule protrudes from the yolk. The eggs are heavier than water, and lie with the oil sphere uppermost and the germ lateral. In one individual sixty-one eggs were found, in another seventy. The exact number in the other two I cannot give, but the number does not differ greatly from the above. From one side of one I took thirty-five eggs, from the other individual an uncertain number. The remaining eggs were left in the gills to develop, but all those that were not subsequently preserved finally died.

The female with eggs can readily be distinguished by her distended gills, and since dead eggs become opaque, such can readily be distinguished through the translucent opercles and branchiostegals. Dead eggs are retained in the gill cavity till they disintegrate.

I have never secured as many young from any female as eggs enumerated above. This may have been either on account of the dying of many eggs or the liberation of the young during the struggle of capture.

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[1] This is the first of a series of embryos obtained through a grant of $100 from the Elizabeth Thompson Science Fund, made to enable me to continue my work on the blind-fishes.

The details of the embryology are now under consideration. Emphasis need be laid only on the fact that Amblyopsis is *not viviparous*, and that the breeding period extends at least from the first of March to November, and probably throughout the year. A female with nearly ripe eggs was secured on September 9, and since these would have been carried, either as eggs or young, for about two months longer, November is a safe limit. Between November and March but one trip has been made to the caves, and this does not permit of a final settlement as to whether or not breeding is carried on at this time.

Certain structures gain an entirely new significance in the light of the breeding habits. These are the enlarged gill cavities, with the small gills, the closely applied branchiostegal membrane, and the position of the anus and sexual orifices. The latter are placed just behind the gill membrane, in such close proximity to it that they can be covered by it. It is probable, therefore, that the membrane is drawn over the sexual orifice and the eggs deposited directly into the gill cavity. In an individual 35 mm. long the anus is situated between the origin of the pectorals; in one 25 mm. long it lies between the pectorals and ventrals. In the young it lies behind the ventrals, as in other fishes.

In an aquarium containing six Amblyopsis two took a great antipathy to each other and engaged in frequent contests. Whenever they came in contact a vigorous contest began. Frequently they came to have a position with broadside to broadside, their heads pointing in opposite directions. At such a time the fight consists in quick lateral thrusts toward the antagonist to seize him with the mouth. The motion is instantly parried by a similar move by the antagonist. This blind punching may be kept up for a few seconds, when by their vigorous motions they lose each other and jerk themselves through the water from side to side, apparently hunting for each other. At this time they are very agile and move with precision. When the belligerents meet one above the other, the snapping and punching are of a different order. While jerking through the water immediately after a round, if one of the belligerents touches one of the neutrals in the aquarium, it frequently gives it a punch, but does not follow it up, and the unoffending fellow makes haste to get out of the road, the smaller ones doing this most quickly. If after an interval of a few seconds a belligerent meets a neutral, they quietly pass each other without paying any further attention, whereas, if the two belligerents meet again, there is an immédiate response. Whether they recognize each other by touch or by their mutual excitability I do not know. At one time, in another aquarium, I saw one belligerent capture the other by the pectorals. After holding on for a short time it let go and all differences were forgotten.

The thrust is delivered by a single vigorous flip of the tail and caudal to one side.

These fights were frequently noticed, and, as far as determined, always occurred between males.

The absence of secondary sexual differences in the cave fishes is a forcible argument in favor of sexual selection as the factor producing high coloration in the males. The absence of secondary sexual differences in caves opposes the idea of Geddes and Thomson that the differences are the external expression of maleness and femaleness.

Attempts at acclimating Amblyopsis in outside waters have largely failed. A few were placed in Turkey Lake, Indiana. They were surrounded by a fine wire net to keep off other fishes. They died in a few days as the result of attacks of leeches, Saprolegnia, or fish mould, and from unknown causes. Others were kept in an elongated box sunk into the ground where fresh spring water flowed through it constantly. Saprolegnia sooner or later destroyed all of them. They live longest in quiet aquaria where the water is rarely changed. The young I have secured died, with one exception, within a few weeks. The difficulty of rearing the young is not at all insurmountable. They eat readily. Their aquaria must be kept free from green plants and have a layer of fine mud with a few decaying leaves in the bottom. They will feed on minute crustaceans and other micro-organisms. When they have reached a sufficient size, Asellus are greedily devoured. Fish mould is the bane of the larvæ. Many of them were found with tufts of the hyphæ growing out of their mouths and gill openings.

A colony has been apparently successfully planted in an open pool at Winona Lake, Indiana.

III. *The Color of the Amblyopsidæ.*

The three species of Chologaster are colored with varying intensity from C. cornutus, in Florida, which is darkest, to C. agassizii, in Mammoth Cave, in which the color is faintest. The color cells are in all cases arranged in a definite pattern. These are determined by the underlying muscles. The pattern consists of three longitudinal bands on the sides following the line where the muscle segments are angularly bent, and cross stripes along the line separating successive segments.

The lower side of the head and the abdomen of Chologaster papilliferus are sparingly pigmented and translucent. The underlying liver and gills give the parts a rosy tinge. On the sides and top of the head, pigment is abundant. There is a more densely pigmented area extending along the middle of the back, beginning as a narrow stripe at the nape, and widening to the dorsal fin behind, where it occupies the entire back. On the sides are three narrow stripes, which, owing to the accumulation of pigment in two layers, are quite dark. Each stripe has a lighter central band, widest at the middle of the sides. The light band, without the conspicuous bordering dark stripes, runs along the middle of the belly. The sides are thickly covered with a layer of pigment, leaving usually colorless lines where connective tissue separates successive myotomes. On the sides of the tail the pigment is dense on either side of these colorless lines. A dark band extends along the sides of the head through the eye. The top of the head is dark.

The pattern of Chologaster cornutus agrees with that of papilliferus. The longitudinal bands are much darker and wider and without the light central streak. The middle band is much wider than the others, and is continued forward to the tip of the snout. The amount of color present varies very greatly with the locality from which the specimens come.

The general color of C. agassizii is light gray. The scales are lighter than the area surrounding them.

The color pattern is more striking than in the other species of the genus. Each somite is bordered by a dark line. The lines of successive somites are separated by an almost imperceptible colorless line. A broad, not sharply defined, band extends along the sides. The middle of this is lighter than the margin. Another one extends between the somites and the ventral musculature, another from the nape between the lateral somites and the dorsal muscles, and a diverging one from near the nape to either side of the dorsal fin. Dark areas are found along either side of the dorsal and anal fins. These areas are caused by the accumulation of pigment along the borders of the small muscles of the fins. Still another dark area is found about the caudal. The ventral surface is white, except the accumulation of pigment along the lines separating the muscles.

The fins are uniformly light gray. A light area is found on both the upper and lower part of the caudal peduncle, just within the first short rays of the caudal.

The general color of Typhlichthys is cream and pink. It is abundantly pigmented. In younger specimens the pigment is arranged in definite areas about the head. In the old it is more uniformly distributed, being, however, specially abundant about the brain. The pigment pattern of the body is precisely as in Chologaster, except that the individual pigment cells are minute and their aggregate not evident except under the lens.

The retention of the color pattern of Chologaster in Typhlichthys is not less interesting than the retention of similar habits. It is perhaps due to different causes. The color pattern in Chologaster is determined by the underlying muscular structure, and the retention of a similar pattern in Typhlichthys is due to the same underlying structure, rather than to the direct hereditary transmission of the color pattern.

In Amblyopsis the color is much less marked than in Typhlichthys. They are flesh-colored, ranging to purple in the gill region, where the blood of the gills can be seen through the overlying structures, and over the liver, which can be seen through the translucent sides and ventral wall. About the head and bases of the fins the color is yellowish, resembling diluted blood. The surface of the body is slightly iridescent, and the surface of the head has a velvety, peach-bloom appearance.

The general pink color of Amblyopsis is due to the blood. It is not due to any abnormal development of blood vessels in the dermis. In the fins, where the blood vessels are near the surface, the general effect is a yellowish color. The surface vessels of the dermis also appear yellowish. It is only on account of the translucent condition of all the tissues, permitting the deeper vessels to show through a certain thickness, that the pink effect is produced. Amblyopsis has always been spoken of as white. The term "white aquatic ghosts" of Cope is very apt, for they do appear white in the caves, and their gliding motion has an uncanny effect. All alcoholic specimens are white.

The chromatophores in Amblyopsis are differentiated and contain color before the yolk is absorbed. The black chromatophores are minute granules, few (fifteen or thereabout) to the segment. In an older larva the pigment was much more abundant. The eyes are pigmented early, — shortly before hatching, — and, owing to their pigment, they soon become conspicuous and remain so till the fish has reached 50 mm. in length, when the overlying tissues have become thick. The pigment of the body is lost, or, what amounts to the same thing, does not increase much with age. There is an abundance of pigment cells in the adult, but they are very poor in pigment, and, being in the dermis and covered by the thick layer of epidermis rich in glands, are not apparent. Pigment cells are also abundant in deeper tissues in the adult, so that, while no pigment is visible on the surface, an abundance of chromatophores are present in deeper tissues.

The pigment cells cannot be made to show themselves even by a prolonged stay in the light. The old, if kept in the light, will not become darker, and a young one reared in the light until ten months old not only showed no increase in the pigmentation, but lost its pigment, taking on the exact pigmentless coloration of the adult. Pigment cells are late in appearing in Amblyopsis. When the young are two months old, pigment is abundant. This pigmented condition is evidently a hereditarily transmitted condition. It disappears with age. Primarily this disappearance was probably individual. But, as in the flounder, the depigmentation has also become hereditarily transmitted, for even those individuals reared in the light lose the color.

Numerous facts and experiments show that, while pigment may be and is developed in total darkness, the amount of color in an individual animal depends, other things equal, directly on the amount of light to which it is habitually exposed.[1]

The lower and upper surfaces of the flounder, the one protected and the other exposed to the light, give the most striking example, and the argument is clinched here by the fact, noted by Cunningham,[2] that a flounder whose lower side is for long

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[1] A number of apparently contradictory observations may be noted: *a*. The absence of pigment in pelagic animals or their larvæ, which depend on their colorless condition for their existence, is evidently due to causes entirely different from those preventing the formation of pigment in cave animals. Natural selection has, in pelagic animals, eliminated the color. *b*. The expanding of chromatophores, when an animal is over a dark background or in the dark, and the contracting over a light background, which may take place instantly or at the expiration of several days, is evidently also a different question. The observations of Cunningham, Agassiz, and Semper along this line are of interest. *c*. Fischel (*A. M. Anat.*, vol. xlvii, pp. 719–734, Pl. XXXVII, 1893) has noticed that larvæ of salamanders reared in water at 6–7° are dark, while others kept in water from 15–58° are light. *d*. Flemming (*A. M. Anat.*, vol. xlviii, pp. 369–374, 1896) found that with uniform temperature in two vessels side by side, the one dark, the other light, the salamander larvæ in the *dark* vessel develop pigment cells rich in color granules; the larvæ in the white vessels become pale, although the number and character of the pigment cells is not otherwise changed. The difference is entirely due to the character of the vessels, for, if the larvæ are taken from the dark to the light vessel, they become light-colored in a few days. *e*. Semper (Animal Life, p. 89) records that ". . . in the tadpoles of our common toads and frogs the pigment is equally well developed in yellow, blue, or red light, and in absolute darkness." This was to be expected, for even in the young of cave animals pigment is, as a rule, well developed. *f*. Pouchet (*Arch. de Physiol. et d'Anat.*, 1876, and *Rev. Scient.*, vol. xiii, 1897) has demonstrated that change in color cells, such as are mentioned under *b* and *d*, is brought about by the reflex control from the eye. The section of the great sympathetic nerve puts an end to the changes of color under the influence of light.

[2] Philos. Trans., pp. 765–812. 1893.

periods exposed to the light takes on color. Loeb has shown that, in the yolk sacks of Fundulus embryos, more pigment cells are developed if the embryos are kept in the light than when they are kept in the dark. However, in the body, and especially in the eye, the pigmentation was not affected by the absence of light.

The general absence of color in cave animals is conceded. Packard[1] states "as regards change of color, we do not recall an exception to the general rule that all cave animals are either colorless or nearly white, or, as in the case of Arachnida and insects, much paler than their out-of-door relatives." Chilton[2] has made the same observations on the underground animals of New Zealand. Similar observations have been recorded by Lönnberg,[3] Carpenter,[4] Schmeil,[5] and Vire.[6]

Hamann[7] enumerates a number of species living both in caves and above ground. In such cases the underground individuals are paler than the others. This confirms similar observations of Packard.

Poulton[8] has mentioned that Proteus becomes darker when exposed to the light. This has been verified by others. In Typhlotriton, larvæ living at the entrance of a cave are dark, while the adult living further in the cave are much lighter, but with many chromatophores containing a small amount of color. Epigæan fishes found in caves are always lighter in color than their confrères outside.

We have thus numerous examples of colored epigæan animals bleaching in caves and also bleached cave animals turning dark when exposed to the light. We have also animals in which the side habitually turned to the dark is colorless, while the side habitually turned to the light is colored. Finally we have cave animals that are permanently bleached.

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[1] *Mem. Nat. Acad. Sci.*, vol. iv.

[2] *Trans. Linn. Soc. Lond.*, 2d Ser., vol. vi, pp. 163–284.

[3] *Zool. Anz.*, vol. xvii, p. 125.

[4] *Irish Naturalist*, vol. iv, p. 25.

[5] *Zeitsch. f. Naturw.*, vol. lxvi, p. 339.

[6] *Bull. Mus. Natur. Hist.* Paris, 1895, p. 143.

[7] Europ. Höhlenfauna, p. 6.

[8] The Colour of Animals, p. 91.

Natural selection cannot have affected the coloration of the cave forms, for it can be of absolutely no consequence whether a cave species is white or black. It could only affect the coloration indirectly in one of two ways: first, as a matter of economy, but since the *individual* is in part bleached by the direct effect of the darkness, there is no reason why natural selection should come into play at all in reducing the pigment as a matter of economy; second, Romanes[1] has supposed that the color disappeared through the selection of correlated structures, a supposition he found scarcely conceivable when the variety of animals showing the bleached condition was considered.

Panmixia cannot account for the discharge of the color, since it returns in some species when they are exposed to the light, and disappears to a certain extent in others when kept in the dark. Panmixia, Romanes thinks, may have *helped* to discharge the color. In many instances the coloration is a protective adaptation and therefore maintained by selection. "Panmixia might, in such instances, lower the general average to what has been termed the birth mean." Proteus is perhaps such an instance. But in this species the bleached condition has not yet been hereditarily established, and since each individual is independently affected, "the main cause of change must have been of that direct order which we understand by the term 'climatic.'"

Since, however, the bleached condition, which in the first instance is an individual reaction to the absence of light, has become hereditarily established in Amblyopsis, so that the bleaching goes on even when the young are reared in the light, it is evident that in Amblyopsis we have the direct effect of the environment on the individual hereditarily established.

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[1] Darwin and after Darwin.

NINTH LECTURE.

SOME GOVERNING FACTORS USUALLY NEGLECTED IN BIOLOGICAL INVESTIGATIONS.

ALPHEUS HYATT.

THE object of this lecture will be fully attained if, by fragmentary statements, I can attract the attention of my audience to certain neglected or little understood facts and theories.

There will be no attempt to prove any special theory, but simply to make statements and refer interested readers, if I am so fortunate as to have any, to works in which more extended information can be found.

The terminology used in the following pages has been found necessary to express more exact ideas of the relations of phenomena which cannot be accurately described without such aids. An old term used in a new sense is confusing, because the accepted usage invariably clings to the word in the minds of all but the inventor of the new meaning.

It may seem useless to dwell on that familiar word "evolution," but, as a matter of fact, misconceptions of the true meaning of this term are by no means rare, and a correct point of view is necessary, especially in considering the relations of ontogeny and phylogeny.

Evolution is essentially continuity of matter moving through time and space, and actually changing as it moves. The laws of evolution are concise descriptive expressions of the modes in which these movements and changes have taken place. The causes of evolution and of the accompanying changes are still more profound and difficult, and fortunately not pertinent to the immediate object of this lecture.

This definition of evolution is based upon the whole mass of phenomena which has been shifting and modifying itself before the eyes of observers from the earliest times to the present day. The fact of perpetual change is acknowledged, and it has also been generally recognized that different bodies change in variable degrees. Some substances are so elemental and primitive in comparison with those succeeding them in time or in their own series that they can be spoken of as relatively unchangeable in comparison with these. The inorganic body that we now speak of as the ether and some of the chemical elements may be rationally imaged as still existent in unchanged condition in some parts of the universe, and in those particular cases there would be continuity without change, and consequently no evolution in the sense that this is generally used.

It has also been quite commonly and reasonably supposed that the most primitive of the existing forms of the animal kingdom among Protozoa, Protamœba, for example, or even so comparatively complex an animal as the existing Amœba, may still retain most of the characters that they possessed when near the beginning point in the evolution of organisms.

It is difficult, in view of such possibilities, to avoid the theoretical conclusion that the evolution of organisms was quite distinct in its first period, and the earliest primitive organisms necessarily more like inorganisms and comparatively unchanging in their structures. In other words, evolution probably began to work gradually, and there was necessarily a time in which the laws of organic evolution, as stated above, were comparatively inoperative. Biology, like mathematics, has therefore an imaginary zero point in time and space, so far as vital characters are concerned, and an actual unit of departure only in the simplest organisms.

Terrestrial organic bodies necessarily move forwards in time and space with the earth, but they have also definite motions of their own. There are two classes of these: (1) the movements of the single organism through its different stages of growth and development. These ontogenic changes are divisible into five periods — the embryonic or earliest usually encysted or ovarian stages, the nepionic or baby stage, the neanic or adolescent, the ephebic or adult, and the gerontic or senile stage. These pass insensibly into each other and have two phases — the progressive, leading to the full-blown adult, and the retrogressive, leading by senile degeneration to the final destruction of organic continuity in death.

The second class of movements (2) take place on a larger scale, and have been designated Phylogeny. This is the life history of the stock, but is more generally conceived of as the evolutionary history of any series of genetically connected forms.

If the ontogeny be granted as possessing capacity for change and the power of reproduction, the phylogeny becomes necessarily a function or product of the ontogeny. This is so obvious that it would need no explanation but for the fact that at present the appearance of variations in the young is supposed by some to indicate fundamental inherent tendencies of organisms to depart from the parent stock.

The mass of evidence and all the obvious phenomena, however, show the identity of reproduction and heredity; they are both necessarily the production of like by like. The simplest forms of reproduction, the division of the unicellular Protozoa, the formation of colonies among Protozoa, and all the asexual modes of reproduction have this character in the Metazoa. The sexual modes of reproduction do not alter these fundamental relations, except in so far as the spermatozoa graft different elements upon the ovum, which influence the subsequent characters of the organism. The phenomena of alternation of generations in Hydrozoa and the hyper-metamorphoses of insects cannot be cited against the old views of heredity and reproduction, since the cycle of the ontogeny in these invariably swings around to the same form, however great the deviation of the intermediate generations or stages of development.

Variations in any given forms are, as a rule, at first infinitely slight as compared with the vast mass of recapitulated characters. They are necessarily grafts upon stock structures until they become incorporated with them, and then are subject to the same law of reproduction. If the old position is true, that an organism when it evolves into another organism necessarily gives rise to one like itself, then the evolution of a genetically connected stock is an inevitable corollary of the existence of a single organism endowed with the power of reproduction. If phylogeny is a product or function of ontogeny, there is a natural basis for the correlations between these two classes of organic movements, and their essential similarity is probably due to this connection.[1]

While this is obviously true, and one can describe the phylogeny in parallel terms to those used for the ontogeny, as is done further on, we should nevertheless bear in mind certain essential distinctions. Thus, the genetic stock has not the absolute continuity of the ontogeny. When regarded in any living or fossil fauna it is an aggregate of organisms, a mosaic of single life histories as distinctly separated from each other in time and space as the pieces of mosaic, except during the brief stage of parental connection. All the changes and movements in phylogeny are necessarily based on two conditions: first, the aspects of the faunas on any one level in time, and second, the relations of series of these on successive levels in time. The successive faunas on different levels, as shown by the geologic history of any genetically connected stock of phylum, resolve themselves into very definite series of pictures characterized by distinct structural and physiological changes.

Both the ontogeny and phylogeny start from the same initial point, and the divergencies necessarily take place first in the ontogeny and are afterwards transmitted to the phylogeny. This initial point for the organism is of course the Protozoan-

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[1] Jackson, in his "Localized Stages in Development of Plants and Animals," *Mem. Boston Society of Natural History*, vol. v, No. 4, 1899, has lately added another chapter to the correlations of ontogeny and phylogeny in showing that the twigs and leaves of plants and plates of the echinoderms, or inflection of the septal edges (sutures) of an Ammonite, or bud of an Actinozoön, etc., although arising from or in the body of an adult organism, repeat the ontogenetic metamorphoses or changes which occurred in similar parts that first arose in the early stages. He has also applied this newly discovered phase of the ontogeny to the translation of the phylogeny and the natural classification of groups.

like form recapitulated in every ovum more or less; but leaving this remote region on one side, we find the initial point for any branch, large or small, of the animal kingdom is very often a primitive form, acquiring the characters of its successors late in life; or they may have them as yet in such indefinite shape that they become perceptible only after this primitive ancestor has been traced to better characterized descendants through the gradations of intermediate forms. Several phyla have been followed out by the painful and tedious process of tracking from species to species, and others by using the ontogeny or life histories of different forms as indices of the natural relations of groups.[1]

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[1] The following is a partial list of these:

BEECHER. Rev. of the Families of Loop-bearing Brachiopoda. *Trans. Conn. Acad. Sci.* Vol. iii, 4th Ser., 1893, pp. 376–391.

BEECHER. Outline of a Natural Classification of the Trilobites. *Amer. Journ. of Sci.* Vol. iii, 4th Ser., 1897, pp. 86–106 and 181–207.

BEECHER. Trilobites in *Zittel's Text-Book of Paleontology*, Am. Ed.

BUCKMAN, S. S. Mon. Inf. Ool. Ammonites. *Paleontogr. Soc.* London, 1887, and still in course of publication.

HILGENDORF. Planorbis multiformis in Steinh. Süsswasserkalk. *Monatab. der Preuss. Acad. der Wiss.*, p. 474. Berlin, July, 1866.

HYATT. Genesis of Tertiary species of Planorbis at Steinheim. *Ann. Mem. Bost. Soc. Nat. Hist.* 1880.

HYATT. Genesis of the Arietidæ. *Smith's Contr.?* No. 673, 1889, and *Mem. Mus. Comp. Zoöl.*

HYATT. Genera of Fossil Cephalopoda. *Proc. Bost. Soc. Nat. Hist.* Vol. xxii, 1883, pp. 253–338.

HYATT. Phylogeny of an Acquired Characteristic. *Proc. Amer. Phil. Soc., Philadelphia.* Vol. xxxii, No. 143, 1894.

HYATT. Cephalopoda in *Zittel's Text-Book of Paleontology*, Am. Ed., in course of publication.

JACKSON. Phylogeny of Pelecypoda. *Mem. Bost. Soc. Nat. Hist.* Vol. iv, No. 8, 1890, pp. 277–400.

JACKSON. Studies of Palechinoidea. *Bull. Geol. Soc. Amer.* Vol. vii, pp. 171–254.

NEUMAYER. Beit. z. Kennt. foss. Binnen-Fauna. *Jahrb. Geol. Reichs.* Bd. xix, No. 3, 1869, pp. 355–382.

SCHUCHERT. A Classification of the Brachiopoda. *Amer. Geol.* Vols. xi and xiii, 1893–94.

SCHUCHERT. Brachiopoda in *Zittel's Text-Book of Paleontology.* Vol. i, Pt. I, 1896.

WÜRTENBERGER. Studien über die Stammesgeschichte der Ammoniten. *Darwinistische Schriften.* No. 5. Leipzig, 1880.

In these as well as by the general experience of paleobiologists in tracing groups by more empirical methods, phylogeny has been found to be at first progressive. It has, like ontogeny, a period of differentiation, when it spreads out into its many different modifications, called species, genera, and so on, and thus finally attains an acme of progress. This acme is followed by a phase of retrogression in genetic series that have what may be called a complete cycle, and this ultimately ends in extinction. All genetic series do not have complete cycles, any more than all individuals have complete existences, and some certainly possess introduced adaptive stages (Echinodermata, Insecta) that complicate the record. Reference is here, therefore, confined to those genetic stocks that did pass through both progressive and retrogressive series of changes.

There are other qualifications to such a brief statement that cannot be discussed here, but in a general way it may be said that this hypothesis of the correspondences of ontogeny and phylogeny has been worked out by hard labor of minute comparison and the study of definite morphic modifications. The method thus elaborated has been and is now in constant use by a number of paleobiologists, and has proved in application more helpful than its most enthusiastic advocate had hoped. The correspondences are not simply between the youngest stages and the remote ancestors, like those traced by embryologists, but between all the stages of the ontogeny and all the periods and phases of the phylogeny. The recapitulations of the embryonic stages are in fact of no greater importance in these researches than those of the epembryonic or later stages, meaning by this term to include not only the young but also the gerontic or senile metamorphoses. The nepionic stages have characters derived from less remote ancestors than the embryonic; the neanic are similarly related to still nearer progenitors; the ephebic stages give the differentials elaborated in the ontogeny at the acme of the evolution of the stock.[1] Definite periods in the phylogeny characterized by

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[1] "Bioplastology and the Related Branches of Scientific Research," *Proc. Bost. Soc. Nat. Hist.*, vol. xxvii; "Phylogeny of an Acquired Characteristic," *Proc. Amer. Phil. Soc., Philadelphia*, vol. xxxii, Nos. 143, 144, pp. 59–125.

structural details have been worked out and found to correspond to those of the ontogeny, and several authors now describe these different stages of the phylogeny by the use of compound terms derived from phylum and the name of the corresponding stage in the ontogeny. They are in this way enabled to designate the primitive types of the phylum as phylembryonic, the nearer ancestors as phyloneanic, and the full-blown acme as phylephebic.

But, as stated above, this does not finish the history of correlations. Taking up next the retrogressive phase of the ontogeny, similar methods have shown that the retrogressive stages in the evolution of the phylum correspond to those that occur in old age or the gerontic stage, and by a similar terminology we have been able to describe the retrogressive stages of the phylum as being phylogerontic.

There are, however, very essential differences, in the behavior of the correspondences of the ontogeny and phylogeny, between the progressive and retrogressive phases. It will at once strike most thinkers that progressive changes in the ontogeny take place, as a rule, before the reproductive period, and have consequently a more direct influence upon the phylogeny. Their recapitulation in the ontogeny may therefore be caused by heredity, while the recapitulation of the retrogressive characters, which occur in the phylogeny after this period is passed, may perhaps not be caused by heredity, but may be due to the necessarily similar character of degenerative changes in similar organisms having a common origin.

Biologists have not as yet investigated the limits of the reproductive period in the different stages of development of animals, and it is therefore not practicable to solve this question. Who knows, for example, what the limit of the reproductive stage is in a clam or a lobster or any of the invertebrates? That these have senile changes is easily seen, but whether the gerontic stage begins before or after the power of reproduction is lost, if it ever is in the ontogeny of some of these animals, no one seems to know. Certainly in many invertebrates, and in as high a type as Mammalia, senile degeneration sets in long before the reproductive power has disappeared.

Whether phylogerontic characteristics are inherited or simply have a common origin, the facts of correspondence remain the same, and this enables us to speak confidently of the whole cycle of the ontogeny or ontocycle as more or less exactly parallel with the cycle of the phylogeny or phylocycle.

Even in such general statements it is perhaps necessary to caution readers that the retrogressive phases above mentioned are entirely those that apparently occur first in the gerontic stages of the ontogeny of more or less progressive forms of the same genetic stock. There are in some groups, especially among parasites, also introduced, adaptive stages of a retrogressive nature that are not in any sense gerontic, and these are not included in these statements.

Connected organisms, according to such views, are looked upon as complex associations of series diverging like the branches of a tree, each branch or phylum passing through a stage of phyletic rejuvenation represented by the more or less primitive forms with which it begins. These radicals are more or less closely related or divergent as compared with the common radical stock or trunk, according to their position in time or their grade in natural classification, but become in turn starting members of new series. These series, if they take a normal course, first progress in structure and then retrogress, but ever proceed onwards in time and space until extinction closes their career.

All naturalists, even at the present time, have not acquired the habit of looking upon organisms as in continuous motion and unceasingly changing while moving onwards through time and space. Farlow treats the errors due to this omission in his paper, read before the Boston meeting of the American Association for the Advancement of Science in 1898, on "The Conception of Species as affected by Recent Investigations on Fungi." This high authority writes as follows: "Our so-called species are merely snapshots at the procession of nature as it passes along before us. In any case it represents only a temporary phase, and in a short time will no longer be a faithful picture of what really lies before us, for we must not forget that the procession is moving constantly onwards, and at a more rapid pace than some suspect." The errors caused by the want of a full realization of this point of view consist largely in sins of omission, as shown by the absence of accurate records. For example, every observation on a species should be dated, location, temperature given, etc., and all the relations, as far as practicable, described, such as habits, food, number of specimens collected, etc. The observations of marine naturalists, under the leadership of Verrill, have reached a high degree of accuracy in these respects, while those of terrestrial faunas have left much to be desired in some departments, especially Mollusca.

Observers in all departments, as a rule, have also neglected the obvious manifestations of senility, and treated all animals except man as if they were exempt from growing old. Apparently, so far as current descriptions go, the invertebrates and most vertebrates are not affected by senile changes, but simply cut off at the end of their uneventful lives by sudden death, unheralded by any preliminary metamorphoses. Thus conchologists have, for the most part, collected and studied the largest shells with so-called perfect apertures, and described these without any suspicion that they were often dealing solely with extreme senile substages. In fact they have often insisted that these alone were the full-grown representatives of the species, and even in many cases have discouraged the collection and study of the real adults and the younger stages, calling them "immature specimens."

Such views also enable the observer to see at once the fallacy of classifications and diagrams of the relations of organisms that represent them as converging. They diverge from their points of origin, but by no stretching of facts or of the imagination can they be accurately represented anywhere as converging. The series may be parallel, and different genetic series may contain closely representative forms that may have been once considered as belonging to the same genus or even species,[1] but the term "convergence" conveys a false impression. From the genetic point of view no convergence is possible

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[1] See Cope, Origin of the Fittest (New York, 1887), and other essays; also Hyatt, "Arietidæ," *op. cit.*

except in cases of actual "hybridity." This use of "convergence" is an excellent example of a well-meaning but mistaken attempt to convey new ideas by the use of old terms, since I imagine that the authors using this term never meant to imply that such forms were genetically convergent.

Any form of diagram or expression that describes organisms as masses or lines radiating in every direction from a center in order to emphasize the fact that many of them are reversions or returns of preëxisting forms, can be objected to on the same grounds. Suppose the author of such a diagram or description to be correct, and that his so-called reversions are real reversions and not simple parallelisms, nevertheless his reversionary forms sprang necessarily from a given level of time and space, and it is as erroneous as it is unnecessary to represent them by lines or names which are placed below this level. Whatever diagrammatic or descriptive form may be selected it must have a base level in time or space upon which the primal point or points or line or base rests, and below which lines or figures representing genetic stocks should not be drawn.

The serial nature of all organisms and genetic stocks is also thrown into proper prominence by this view of the phenomena of evolution. All series begin necessarily with an imaginary starting point, to which the series may be traced by its gradations, but in which its essential characters do not exist, this being the zero point of that particular series. Such organisms are well known to all phylogenists, animals that would certainly be placed in different series from those to which they are referable through intermediate gradations, if those gradations had not been traced. Such forms often give rise to endless controversy unless they occur associated in the same fauna with the forms into which they grade. Next to this first more or less indefinable beginning comes the simplest unit of the morphic series in which the type is distinctly recognizable. This originates quickly and is followed by a comparatively sudden expansion into a number of distinct and often widely separated forms having very different morphic characters. Thus, the foundations of the various stocks or branches of the phylum are laid by quick processes of evolution, not slowly, as generally supposed.[1] Not only is this true for the smaller phyla of animals, so far as known, but the now well-ascertained fact that most existing types arose during earlier Paleozoic time shows that it is probably a general law for the whole of the animal kingdom.

This excessively plastic period of evolution is followed by a phase of slow changes in which the different branches of the phylum acquire their slighter and less marked divergencies. Thus, evolution is not invariable, but moves with extreme slowness while each branch or phylum is in the middle age or acme of phylogeny. Finally, when retrogression begins, the pace is again quicker, and the differences greater, owing to the abrupt losses of progressive characters, and sometimes to the sudden introduction of new and startling modifications.

Many types, even at the present time, are still in the progressive stage of advancement, but fortunately the most specialized and complex of all types, the Mammalia, has nearly, if not quite, reached an advanced phase of retrogression, and is an admirable example of the quicker elaboration of differences and the change of law in highly retrogressive types.

The record of the immediate ancestor of man is either lost or so difficult to trace in the embryo and younger stages that observers have failed to come to any agreement, and yet he is plainly descended from some simian predecessor. He is not distinct from the most specialized of this group, the chimpanzee or gorilla, either by his bones or soft parts, so that in the absence of his works a paleobiologist would not separate him widely from them. All efforts to demonstrate adequate structural characters for the separation of man from the anthropoids have failed. The size of the brain cannot be admitted as adequate, or any other differences of mass. The wide gulf between man and his nearest relatives nevertheless becomes plainer with every effort to close it up, and it is obviously physiological. Even if all the intermediate links between him and some anthropoid or other simian ancestor were to be found, as they probably will be in time, the differences would remain just as marked as before.

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[1] "Phylogeny of an Acquired Characteristic," *op. cit.*, pp. 366–371.

The evidence of his vast physiological powers, as shown in his works, are in obvious correlation with his progressive characters, such as the upright position and all the correlated modifications like the sigmoidal outline of the back, the huge bipedal legs, the differentiation of the hands and feet. The obvious correlation of his works also with the size of the brain and shortening up of the lower face, and other retrogressive characters, as they are correctly diagnosed by several authors, notably Cope and Minot, make up a paradox which is insoluble as long as evolution is looked upon as uniform in its action.

In types terminating phylogenetic series, however, such intermixtures often occur on a smaller scale. These are not generalized, but highly specialized creatures, and notwithstanding their retrogressive modifications, they are necessarily the highest in position or grade, as determined by the facts of succession in time and genetic position. Man, like one of these terminals, is retrogressive in essential structures, and, like one of these also, is separable from his nearest congeneric relatives, through the change of law which has converted morphic retrogression into physiological progression. The brain, although larger, is, when compared with that of the human embryo and those of the young of other mammals, plainly arrested in development, as demonstrated by Cope and Minot. It maintains, in its proportions to the mass of the body, the general characters of the embryos of the Mammalia, but has nevertheless differentiated in intimate structure and evolved into an organ of such enormous and novel powers that its functional work has become distinct qualitatively, as well as infinitely greater quantitatively, than that of the animal brain.

The disregard of researches in comparative ontogeny and phylogeny has also led most investigators of the Darwinian school to believe in the uniformity of action of the laws of evolution, an error that has had far-reaching consequences. Thus Poulton, recognizing that the sudden appearance of animal types in geologic strata was opposed to the Darwinian theory of the slow accumulation of differentials through natural selection, and in order to reconcile the facts with this theory, has assumed that there must have been in existence a vast series of faunas which preceded the known Paleozoic, and in which the earliest known fossil faunas were slowly brought into being. The objections to this hypothesis are, not only that evolution probably proceeded with greater rapidity at first, and that in every period the first coming in of types was sudden and in accordance with the law of phylogenesis stated above, but, what is still more convincing, the researches of explorers do not sustain this position. The great thickness of unaltered rocks found in the lowest Paleozoic of the Colorado Cañon, and hunted over step by step by C. D. Walcott, whose capacity for finding fossils in the field has perhaps never been surpassed, and the researches of C. F. Matthews, an equally careful field worker, in the Cambrian of Nova Scotia, and Walcott again in Newfoundland, as well as all other observers in other lands, have failed in discovering any confirmations of the supposed former existence of preliminary faunas of the extent required by Poulton's theory. If Darwinians had not only looked upon animals theoretically as genetic series, but put their views into practice and worked out examples of phylogenesis, they would long since have ceased to regard the uniformitarian hypothesis as tenable.

The disregard of such researches will, I venture to suggest, be found to affect all work upon the histology and embryology of living animals. This must be the result if the theoretical position taken in this paper be true, or even true in so far as the nature of series is concerned. If series, for example, have not been elaborated by uniform law, but by a process that is forever changing its rate, and tending, as in the case of highly retrogressive forms, like man, to depart so widely from the parent type as to make their connections very difficult to determine, it follows that all inferences with regard to phylogeny not based upon the comparative study of genetic series are more or less likely to lead to erroneous conclusions.

Perhaps the only researches in the embryology of existing animals that may be said to have been followed out in accordance with this view are those that have described the serial relations of cells in the ontogeny and have refrained from drawing phylogenetic conclusions, except perhaps the most general, from the data thus afforded. There is, for example, no conclusive evidence of the inference that the bodies of all Metazoa are to be considered as a connected mass, and not as a colony of cells, because the cells of the bodies of the more complex invertebrates and vertebrates have been found to have such close protoplasmic connections that no cellular independence of action is possible. The highly differentiated tissues of complex structures which stand high in the gradations of any genetic series might possess in every case examined the characters that have been relied upon to disprove the cell theory, and yet the simple primitive forms of the same genetic series have less closely connected and more independent cells even in their full-grown stages.

Who knows, also, what the closer study of the ontogeny may bring out with regard to the connections of cells, especially in the building up of the earliest stages among the simplest forms of each great group? There is, it appears to me, no solid objection in embryology to the old theory of the rise of the Metazoa out of Protozoa by a series of gradations that are more or less distinctly traceable in the embryology of the Metazoa, especially if the position taken by the lecturer is true, that Volvox and Eudorina of the fresh waters is an intermediate type, a real mesozoön, standing between the Protozoa and Metazoa, and representing in their permanent aspect the transient, single-layered blastula of the true Metazoa.[1]

Even leaving this out as inadmissible does not vitiate the view taken by the advocates of the cell theory so far as the obvious morphic phenomena are concerned. The evidence that the earliest stages of the ovum in the most primitive forms of the different classes of the animal kingdom, as a rule, recapitulate the essential characters of the more primitive Protozoa, or, in other words, possess a reminiscent protozoanal, unicellular stage, seems macroscopically and microscopically demonstrated. In order to destroy the evidence of this obvious morphic basis, it would be necessary to prove that the ova

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[1] "Larval Theory of the Cellular Tissue," *Proc. Bost. Soc. Nat. Hist.*, vol. xxiii, 1884, p. 151. Also "Values in Classifications of Stages of Growth and Decline," etc., *Am. Nat.*, 1888, p. 872.

of selected primitive forms were not homologous with the unicellular bodies of the simplest Protozoa.

I say selected, meaning thereby that an ovum taken at haphazard anywhere throughout the animal kingdom would be as likely to confuse the observer striving to solve the question of the origin of metazoanal structures as to throw light upon this problem. If the students of comparative ontogeny are correct, an organism taken from among the primitive forms of any genetic series has within itself a record of historical value proportioned to its place in its own genetic line and also to the position of its genetic stock in the whole animal kingdom, and its evidence may be, and practically is, dependent upon its grade or position. If it lie near the genetic base of origin, it contains a more complete record, and recapitulates more ancestral characters than if it is drawn from the acmatic period of the phylogeny; and finally, if it come from the other extreme of the phylogeny, where animals are passing through retrogressive phases, its ontogeny may be so deficient as to fail to recapitulate, as in the case of the young of man, the hitherto essential characters of the forms from which it was evolved.

This is the result of a law of development of the ontogeny, which Cope during his life was in the habit of demonstrating and using among recent animals, and the lecturer among fossils. It is particularly well shown in such complete series as may be found in the living Batrachia and in fossil Cephalopoda, and has been independently discovered by several observers.[1]

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[1] The following seems to the author a fair statement of the history of this law. Haeckel, in his Morphologie der Organismen, vol. ii, 1866, p. 184, gave, under the title of "Gesetz der abgekürsten oder vereinfachten Vererbung," what seemed to me for some years to be a definition of this law, but in writing my Bioplastology I came to the conclusion (p. 78) that this was merely an emended general statement of the law of correlation between the ontogeny and phylogeny. An abbreviated statement of the literature seems to be as follows: Hyatt, "Parallelism between Stages of Life of the Individual and Group of Tetrabranchiata," *Mem. Bost. Soc. Nat. Hist.*, vol. i, Pt. II, read Feb. 21, 1866, published 1867 (see Rep. of Custodian of B. S. N. H., May 1, 1867, and May, 1868); Cope, "Origin of Genera," read October, 1868, published 1869 (see Account of Cope's Origin of the Fittest, p. vi, Preface); Würtenburger, *op. cit.*, p. 28, Leipzig, 1880; Buckman, *op. cit.*, vol. for 1891, p. 290, also "Some Laws of Heredity and Their Application to Man," *Proc. Cotteswold Nat. Club*, vol. x, Pt. III, 1891–92, p. 258; Ganong, W. F., "Contr. Morph. and Ecol. of the Cactacæ," read before Society for Plant Morphology and

It has been called the "law of Tachygenesis, or accelerated development," and is known as an isolated fact to most embryologists under the name of "abbreviated development." It is constantly noted by investigators, but, in consequence of their failure to follow out their observations in serial succession according to the natural order of phylogeny, they have not realized that extreme cases of abbreviation are only exaggerated phases of a law of heredity acting more or less in all forms.

In tracing phylogenesis by the correspondences of the younger, stages and by the gradations of the adult stages, it has been found that successive, genetically connected forms tend to inherit the characters of their ancestors at earlier stages than those in which they first appeared in those ancestors. The law as observed by breeders and formulated by Darwin for existing animals and plants is that characters of parental forms tend to be inherited at the same time or earlier in descendants. In phylogeny the result of this is cumulative, and in favor of continuously increasing acceleration. Thus, the latest acquired characteristics of any ancestral form appear earlier, and ever earlier, in its descendants. In this way room is made for still other acquired characters that are introduced later in the lives of these same forms. In its normal course in phylogeny a characteristic takes up in succession different places in the early ephebic, then passes into the neanic substages, and finally comes into actual contact with the more invariable nepionic characters. Then, according to the law of Tachygenesis, it is, as a rule, crowded out of the ontogeny and ceases to be recapitulated. Sometimes, as shown in a yet unpublished paper by Grabau, among Gasteropoda an apparently trivial character may pass this ordeal and actually invade the later substage of the embryo. As a rule, however, the characters that arise in the smaller phyla perish out of the ontogeny when they meet the more persistent nepionic and embryonic characters.

Physiology, Dec. 29, 1897, published in *Ann. of Botany*, vol. xii, December, 1898; and Jackson, "Localized Stages," *op. cit.*, p. 138 (read Nov. 2, 1898, published April, 1899), notices this law in plants. These all, except Jackson's, represent independent rediscoveries of the law of Tachygenesis.

The nepionic or baby stage has been found to have characters derived from the more remote ancestors of the group or general phylum of the class. It may be characteristic of a large genetic division or order, or sometimes, perhaps, what many systematists would call a class or subclass. These nepionic characters often refer us back to earlier geologic time, or even to forms of still unknown faunas. This brief statement is of course very defective, but it is true in a general way and is in accord with the common belief in the comparative invariability of embryonic characteristics which immediately precede the nepionic in the ontogeny. Whether this comparative invariability of earlier ages, which forms the basis of most embryonic conclusions, be considered as due to the similar conditions that may be supposed to guard the earliest stages of every embryo among Metazoa, or whether it be due to heredity, which necessarily recapitulates the processes of rejuvenation derived from the remotest ancestors more faithfully than those later acquired, cannot be discussed here. It is, however, quite plain that embryonic characters, as a rule, possess persistence in direct proportion to their age, the most ancient being usually the most persistent.

The few examples of later acquired characters that have been traced back through earlier and earlier inheritance to the embryonic stages are positive so far as they go, and enable us to see that the variations that do take place in these earlier stages are not the result of a different law from that which governs the inheritance of later acquired characters, but probably the same. It is well known that in the same class, or even order, embryos of the same genetic stock may pass from the detailed representation of many successive stages in the formation of the three layers, and of the body cavity and other parts (ex. Amphioxus), to a comparatively complex abbreviation of what are supposed to be the same processes, since they have identical results in the development of adults. But so many details have been lost, the blastula and gastrula for example, and other characters added, the food yolk as an illustration, that it becomes difficult to see that they are really modifications of identical modes of development. If one is prepared to try the law of

Tachygenesis as a working theory, it is obvious that these exceptional or abbreviated processes can be considered as due to that law, since it works in the same peculiar way — sometimes with simple and straightforward exactness, but often also with a complex of introduced characters that make its translation very difficult.

A large amount of time has been wasted in useless efforts to solve problems of phylogenesis by the aid of the embryology of highly specialized and highly tachygenic forms. These may sometimes throw light astern on their own immediate ancestry, but are often too deficient in genetic recapitulation to be used even in this limited way, and are worse than useless in the solution of problems of phylogenesis in which other genetic series are concerned. Examples of this class are numerous, and almost every embryological work abounds with misdirected efforts to use the early stages of such animals either for or against opinions and theories where their bearing has little or no value. This is particularly evident when they are used to show that there are no positive general correlations between the ontogeny and phylogeny, or when they are spoken of subjectively as "falsifications" of the embryological record, as if nature were playing a game of bluff with scientific observers.

This law also forbids the drawing of general conclusions from haphazard investigation, however numerous, broad, and widespread the facts may be, and is consequently opposed to prevailing statistical methods. These assume that, if a large number of embryos be selected at random, the conclusion drawn from their investigation would correct the discrepancies and errors due to what is called insufficient data. The error in such an assumption is that, while statistics apply, if carefully used, to a multitude of living forms of the same stock (and one may safely trust them perhaps in the general meaning of a character among animals of the same age or the same grade), it does not apply to phylogenetic relations of animals of different grades. The relations of these being serial, all observation of a statistical kind covering animals of different grades must be arranged and compared upon the same basis. It seems to the lecturer, for example, that the real relations of the blastula to the gastrula stage should be solved first by observations made strictly within the limits of some one type, and these be checked off and corrected by reference to the accepted or determinable position of each animal in its own genetic stock. Also that no conclusion could be accurately drawn from these data until the different branches of the animal kingdom had been reviewed in the same way, especially those in which, like the Cephalopoda and Vertebrata, the earliest stages are highly tachygenic and also complicated by the addition of a food yolk or other introduced characters.

So far as the position taken in this paper would be likely to affect such questions, a very good argument can be made for the opinion that the gastrulas formed by the bending in of one pole of the blastula are primitive, and other processes are more or less tachygenic forms of this one. Whether this be accepted or rejected does not, however, invalidate the assumption made here, which is that the search for the solution of all questions relating to the study of phylogeny can hope for success only upon the basis of serial comparison, since this is the only method strictly in accord with the principles of evolution and with the methods that have been so efficient in the investigations of the laws of development of the ontogeny.

All of the above remarks tend one way when considered from a general point of view. First, in the study of organisms we are dealing with plastic substances showing definite gradations which can be arranged in their natural radio-serial divergent lines of classification, either as they appear on the present surface, or upon a succession of levels in past faunas. Second, there are notable and essential differences in the behavior and structure of the beginnings of any single genetic series as compared with its middle terms, and marked differences also between these same middle terms and the final or aberrant terms, or branches of the same series. Third, it is also asserted that the difference between the extremes and the means of any one genetic series is proportional and usually so great that the grade and position of an organism must be taken into account in every phylogenetic investigation. The probable penalty of neglecting this is failure in the attainment of theoretically correct opinions, either through absence of data that can be gathered only in this way or by the misleading nature of the unclassified positive evidence.

Another direction in which legitimate criticism can be made of many modern researches has still more fundamental meaning.

Cope always insisted upon the necessity of regarding the immediate reactions of the organism as the proximal cause of modifications. One cannot read his masterly analysis of the phenomena of Bathmism in the *American Naturalist* for 1893, under the head of "The Energy of Evolution," without realizing that he is accurately translating the two categories of the obvious phenomena of evolution in the following passages:

"The term 'energy' is used to express the motion of matter, and the building of an embryo to maturity is evidently accomplished by the movement of matter in certain definite directions." Then, after stating the wide differences between this sort of energy, so far as it can be estimated by its results, and other sorts of energy, known by their physical results upon inorganisms, he divides the organic results into two classes. "The anagenetic class tends to upward progress in the organic sense, that is, towards the increasing control of its environment by the organism, and towards the origin and development of consciousness and mind. The catagenetic energies tend to the creation of a stable equilibrium of matter in which molar motion is not produced from within, and sensation is impossible. In popular language one class of energies tends to life, the other to death."

The term "Catagenesis" is to me objectionable, and paragenesis would be better; but leaving this aside, Cope has hit the exact explanation of the cycle in the ontogeny and phylogeny, and given us a true, broad picture in these few words of the phenomena of life, and made plain that it is distinct from inorganic phenomena because it possesses internal factors that are the direct and immediate causes of modifications.

How many biologists keep constantly in mind this double relationship of an organism to its surroundings, and the possibility that most morphic modifications are complex phenomena, which necessarily take place through action that proceeds from within outwards. The fact that it takes an appreciable time for an organism to receive an impression, and to begin to react by its own efforts in the presence of an irritating substance, or to begin to change in response to new surroundings, and that its corresponding movements and modifications are suitable, shows this with a simple directness not characteristic of other explanations of the origin of variations. It is not intended to defend this theory, which originated with Lamarck, and was subsequently, but independently, restated by Cope and the author of this lecture, but simply to point out some of the inevitable failures of reasoning due to the neglect of the consideration of the internal reactions of organisms.[1]

Experimental biologists are very apt to neglect this factor in their conclusions, and consequently either invert the natural

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[1] The general impression that neolamarckism implies belief in action of the surroundings is true only so far as these act as stimuli, or may be proved to act directly upon organisms. Neolamarckism, or, better named, the Dynamical School of Biology, does not reject the Darwinian hypothesis when the struggle for existence or selection of any sort is recognized as a result of evolution, as a secondary cause tending to the preservation and perpetuation of differences after these have been originated by fundamental causes. The dynamical hypothesis can be shown to be inoperative only by proving that the internal mechanism of motion in organized beings is ineffectual to produce variations. It deals with the immediate origin of variations through internal reactions, and is really open to any explanations of the subsequent conduct and preservation of these that can be proven.

Since this lecture was delivered, I have had the pleasure of receiving a very profound and interesting printed lecture of Dr. C. O. Whitman, on Animal Behavior, biological lecture at Woods Holl Laboratory, 1898. Although he takes up a position adverse to those advocated in this lecture, this author really does seriously consider some of the neglected theories usually omitted by students of existing biological phenomena, or neobiologists, and so far his lecture meets the general criticisms offered here. It appears to me, however, after reading this and other essays, that a large part of the differences between the schools that believe in the inheritance of acquired characters lies in the different definitions of what is meant by acquired. Again it is, perhaps, as stated by Whitman, very difficult to find a character, or instinct or habit, which will meet his requirements among the habits of existing organisms. Habits "having no hereditary basis predisposing to them" (Whitman, p. 314) are as hard to find in existing complex or fixed organisms as characters having no similar basis. My own definition of an acquired character is that it is not present in the more primitive forms of the same phylum, and tends to occur first in a comparatively late stage of the ontogeny (see "Phylogeny of an Acquired Characteristic," *op. cit.*).

order of causation in their conclusions, or give only more or less partial statements of their results.

The most famous case is that of Schmankewitsch's experiments on Artemia and Branchipus, which you will, I know, pardon me for sketching over hastily in order to give weight to this criticism. This Russian worker on one occasion, after the accidental breaking down of the dams that defended the salt pans near Odessa from the uncontrolled influx of the adjoining brackish waters, observed that the flood brought with it *Artemia mulhauseni* in great numbers, and also destroyed *A. salina* of the dense salt water previously occupying the pans. It struck this keen observer that the conversion of *A. mulhauseni* into *A. salina* might be determined by following closely the changes that would take place after the dam was repaired, and the conversion of the outside brackish water into dense salt water was begun by evaporation. He not only followed these changes successfully, but he also carried on a parallel series of experiments in aquaria.

In both the natural and artificial experiments *A. mulhauseni* of the natural brackish waters changed into *A. salina* of the denser salt waters of the pans after evaporation. Then Schmankewitsch, by inverting the process and diluting the salt water in his aquaria, succeeded in not only producing *A. mulhauseni* again, but proceeded farther, and by decreasing steadily the amount of salt in the solution, evolved artificially Branchipus of the fresh waters. The modifications of these crustaceans were mainly produced in the swimming organs, especially the form and hairiness of the end of the abdomen, and also the final appearance of an additional segment in the abdomen of Branchipus. Schmankewitsch at first attributed these modifications wholly to the effects of the proportionate amount of salt in the water as determined by its density, and subsequently appears to have modified this opinion, allowing that temperature and proportionate amount of oxygen contained in water of different densities might have also been factors in producing the variations observed.[1]

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[1] *Zeitschr. f. wiss. Zool.*, vol. xxv, Supplement, 1875, p. 106; and *Ibid.*, vol. xxix, 1877, p. 429.

Brauer of Vienna, accustomed to the study of the adaptation of organs among insects, and the author of this lecture, accustomed to look for internal factors from a more theoretical point of view, agree in criticising these results as defective, because they did not take into account the fact that the amount of salt may have had no direct effect, except in so far as it increased the density of the fluid in which the animals moved. The fact that the modifications took place mainly in the abdomen, and that the increase in hairiness, and in length and size of this part, and variations of the appendages as the water decreased in density, can be just as probably attributed to the extra growth caused by increased efforts necessary for breathing and swimming in a more rarified medium. Whether this objection be true, or the reverse, does not matter here, since it is simply introduced to show the need of considering the organism as a vitalized body which is capable of reacting from within appropriately in response to external stimuli. Experiments that do not take this essential condition into serious consideration are open to similar objections.

In order to prove a physical cause acting wholly and directly from the outside, the internal reactions of the organism should be shown to be passive, as in the cases of chemical reaction. As in Schmankewitsch's investigation, the conclusions are otherwise open to the objection that they have entirely disregarded the consideration of fundamental elements, and that the physical causes they cite have only set in motion a chain of internal reactions, which are the immediate causes of the observed modifications. Under these conditions, also, strong doubts arise as to the efficiency of the physical causes thus demonstrated, since the mode of experimentation does not exclude the possible existence of other causes that might set the chain of internal reactions going in the same or similar directions.

Dr. H. S. Jennings's masterly studies "On the Reactions to Stimuli in Unicellular Organisms" is one of the very few investigations not affected by such criticisms. These clearly establish the fact that a multiplicity of causes may bring about precisely similar reactions on the part of several of the ciliated Protozoa, like Paramecium, and that it is the organization and not the stimulus that determines the functional results. "Certain agents set up a reaction in the animal, the directive features of which depend entirely upon the structure of the organism."[1]

This author's conclusion is particularly interesting both in its originality and its bold denial of the conclusions of other observers, and the support it gives to the opinions expressed in this paper with regard to the necessity of considering evidence of all kinds from the point of view of the position or grade of the animal. Speaking of one of the most highly organized of the Protozoa, Paramecium, with which he has become very familiar by long-continued study, he says: "An animal that learns nothing, that exercises no choice in any respect, that is attracted to nothing and repelled by nothing, that reacts entirely without reference to the position of external objects, that has but one reaction for the most varied stimuli, can hardly be said to have made the first step in the evolution of mind, and we are not compelled to assume consciousness or intelligence in any form to explain its activities." If Jennings is correct, this highly differentiated protozoön is a zero point in the evolution of consciousness. Whether, however, this is due to the essential character of its protozoanal or unicellular structure or to the loss of functional power cannot be asserted until the simpler forms of Protozoa have been studied in the same thorough way and with corresponding tests of their internal reactions.

The importance of these researches, if confirmed and established, can hardly be overestimated, since they open the way for experimental observation upon the evolution of intelligence, and, if found to be true in simpler organisms, will show that automatic habits come first in the evolution of consciousness, and are not, as often supposed, invariably the results of repeated conscious actions.[2]

A number of other recent papers and books have shown that psychology has its obvious place in the study of organisms, but

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[1] "Psychology of a Protozoön," *Am. Journ. of Psychol.*, vol. x, No. 4, 1899, p. 513.

[2] This does not imply that automatism is not a retrogressive result of the constant repetition of conscious actions, but simply that it is also a primitive or antecedent stage in the progressive evolution of consciousness.

the fundamental importance of this science is not suspected by most investigators. Nevertheless, it has been placed in its proper relations by a number of writers, beginning, perhaps, in definite modern form with Ewald Hering's paper, entitled, "On Memory as a General Function of Organized Matter," an address given before the Imperial Academy of Science at Vienna in 1870. Ribot took substantially the same ground in his work on "Heredity," in 1876, and Haeckel also in "Die Perigenesis der Plastidule," 1876, both being ignorant of Hering's previous publication and of each other's results. These were followed by an equally independent restatement by Cope in the *American Naturalist* in 1889, and the lecturer came near making a fourth in this list. Speculations upon the supposed mysterious and indefinable nature of heredity might have had a far more objective basis for the past twenty-nine years had naturalists, whether in opposition or in approval, considered Hering's theory worthy of their attention.

Confusion exists in the minds of many naturalists, arising from the ordinary conception of memory as a function of conscious intellectual effort and as something essentially distinct from automatic action and habits of all kinds. No essential distinctions actually exist, however, since the two grade into each other.

The essential common characteristic of both is repetition; and to represent this and avoid the risk of employing an old word whose application had to be greatly extended, I have in previous publications used the term "mnemism," or "mnemonism," to express the nature of heredity, and "mnemogenesis" for the theory. Before proceeding further in the argument of this question it is necessary to glance hastily at the supposed modes of transmission of characters.

These can be divided into two classes: (1) those theories that depend upon some peculiar substance supposed to have always been distinct from the surrounding forms of protoplasm. These necessarily assume some sort of corpuscles or organic molecules to be the vehicles of transmission. (2) Those theories that regard the germ plasm as not transmitted from body to body but as arising out of the ordinary protoplasm of the young by perpetual rejuvenation and subsequent differentiation, and therefore in no sense a peculiar substance.

Every purely corpuscular theory, meaning thereby the physical transmission of gemmules, biophors, pangenes, or any other supposed organic bearers of characteristics, must not only account for a difficulty as great as that of the camel and the needle's eye, but must also account for putting the numberless characters, derived from the entire caravan of its immediate progenitors and remote wild ancestors and their progenitors, back to the origin of the phylum through the same narrow tunnel.

One has to imagine the corpuscles and all this active circulation and concentration taking place invisibly, and yet requiring visible vehicles of transmission in the minute spermatozoön and nucleus of the ovum. Then he must picture their redistribution over the body of the offspring, the larger number remaining latent until the proper time arrives for them to develop, and then locating themselves and coming out in exactly the right place, or repeating at the right time some tendency or habit of the ancestors.

One is naturally led to ask how these particles acquired their peculiar tendencies which force organisms along predetermined paths of development, reproducing similar successive stages of development with automatic regularity and repeating with more or less precision the permanent stages of their ancestors? In what way did they acquire their wonderful ability to remain latent until they had reached the proper place, and then measure time so as to wake up and go to work at the right moment? The corpuscular hypotheses provide supposititious vehicles of transmission, but they do not explain how or why these move in their apparently predetermined cycles of change.

Nussbaum's and Minot's[1] theory of panplasm or the continuity of germ plasm seems to be a necessity, if the transmission of germ particles or of organic atoms is rejected. Minot's

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[1] Nussbaum, "Differenzirung d. Geschlechts in Thierreich," *Arch. f. mikr. Anat.*, Bd. xviii, 1880, pp. 1–121.

Minot's "Vererbung u. Verjüngung," *Biol. Centralb.*, Bd. xv, 1895; and *ibid.*, *American Naturalist*, vol. xxx, 1896, pp. 1–9, 89–101.

decision that this substance is not a peculiar plasm but a differentiated product of each organism undergoing a process of rejuvenation as each link in the chain of being is forged, seems more in accord with the facts than that which demands a waveless linear transmission of some peculiar substance.

Neither of these theories excludes the conclusion that heredity of the transmission of like by like is a function of the automatic repetition of the same act, or, in other words, identical with what we call unconscious memory; but it is obvious that if heredity can be expressed by the term "mnemism," the greatest difficulties are removed from the path of the theory of panplasm. This last cannot provide of itself any reasonable explanation of the successive stages of development and their recapitulations of ancestral characters, but with the aid of mnemogenesis it can fully explain these phenomena.

Professor Cope has insisted upon the truth of this theory in two essays. The following are quotations from his article "On Inheritance in Evolution":

> If the doctrine of kinetogenesis be true, this energy (the building energy) has been moulded by the interaction of the living being and its environment. It is the expression of the habitual movements of the organism which have become impressed on the reproductive elements. It is evident that these and the other organic units of which the organization is composed possess a memory which determines their destiny in the building of the embryo. This is indicated by the recapitulation of the phylogenetic history of its ancestors displayed in embryonic growth. This memory, perhaps, has the same molecular basis as the conscious memory; but, for reasons unknown to us, consciousness does not preside over its activities. The energy which follows its guidance has become automatic, and it builds what it builds with the same regardlessness of immediate surroundings as that which is displayed by the crystallific growth energy. It is incapable of a new design. It appears to me that we can more readily conceive of the transmission of a resultant form of energy of this kind to the germ plasma than of material particles of gemmules. Such a theory is sustained by known cases of the influence of maternal impression upon the growing fœtus. Going into greater detail, we may compare the building of the embryo to the unfolding of the record of a memory which is stored in the central nervous organism

of the parent, and impressed in greater or less part on the germ plasma, in the order in which it was stored. The basis of memory is reasonably supposed to be a molecular (or atomic) arrangement from which can issue only a definite corresponding mode of action. That such an arrangement exists in the central nervous organism is demonstrated by automatic and reflex movements.[1]

Any naturalist who has studied the sudden recurrence of habits in animals, usually accounted for by the use of the meaningless term "instinct," will find that this is no figment of the imagination, but a working hypothesis that may be used and tested.

Although Ribot's work on "Heredity" has not been widely read or accepted by scientists, perhaps because he admits many facts as substantial evidence which have not been verified, it is nevertheless the most profound work on this subject that has been published. This learned authority, after careful investigation, came to the same startling conclusion that memory was the only function of organisms which could be closely compared with heredity, and he brings forward a large number of facts, to which many others can be easily added. He says "heredity, indeed, is a specific memory; it is to the species what memory is to the individual. Facts will hereafter show that this is no metaphor."

The authors quoted above have not discussed memory, as has been commonly supposed by naturalists, in a metaphysical sense, but as an organic function arising from voluntary or involuntary repetitions of conscious or unconscious actions. Ribot indulged in general speculations and was hampered by them to a notable extent, but he struck the keynote of the dynamical theory of evolution. Thus he says: "Every act leaves in our physical and mental constitution a tendency to reproduce itself, and whenever this reproduction occurs the tendency is strengthened; and thus a tendency, often repeated, becomes automatic. This automatism is the link between memory and habit, and gave rise to the saying that memory is

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[1] *American Naturalist*, vol. xvi, 1882, pp. 454–469; reprinted in The Origin of the Fittest, pp. 405–421, and "On Inheritance in Evolution," *American Naturalist*, vol. xxiii, 1889, pp. 1058–1071.

only a form of habit — a proposition which, with some restrictions, is true."[1]

Repetition, or the reproduction of parallelisms, is equally characteristic of memory and of heredity, nor can either be conceived of as having a tendency to produce variations. It is entirely reasonable that any novel acquired habit, due to conscious effort or to involuntary reactions of organisms in the presence of external stimuli, may be regarded as one of the products of memory. It follows from this that any structural modifications which may result from the repetition of the same acts or habits can, with equal reason, be attributed to memory. The tendency of descendants to perform the same action, *i.e.*, to manifest the same habit in the presence of similar stimuli, when no special structure has been originated, can thus be readily accounted for if one considers heredity to be one form of organic memory, or mnemism.

When special tendencies or structures have arisen, their reappearance in descendants at the same time, or earlier, does not present the same difficulties that it seems to place in the path of other theories, but becomes one of the strongest confirmations of the mnemogenic hypothesis.

In mnemonics it is the machine-like regularity of the succession of cause and effect, of one word begetting the next, that surprises the student, the recurrence in the mind of long-forgotten words, languages, and scenes, either through the recurrence of some inciting cause or upon the removal of interfering causes, as in the recapitulation of youthful reminiscences in aged persons. Mnemotechnics, as embodied in the various systems which have been devised to cultivate the memory, consists essentially in teaching the habit of forming a chain of associated ideas or words leading up to the word or thought to be recalled.

Even reversions that may be supposed to be purely sporadic do not oppose any serious obstacle, since there must always be latent mnemism in the cells and organs ready to manifest itself whenever more recently acquired characters are prevented from being developed in their proper succession. This is the most

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[1] Heredity. Translation. New York, 1876.

frequent form in which reversions are found among Ammonitinæ. There are, of course, difficulties in the path of this hypothesis, such as the so-called premature development of characteristics and some other rare phenomena interfering with the regular recapitulation of ancestral characters in the ontogeny. These at present can only be explained by assuming an irregularity in the action of mnemogenesis, due to disturbing causes, but this, even as a provisional suggestion, is not satisfactory without adequate statement of the nature of these causes in specific cases.

This subject has been treated of at greater length in other publications, and these remarks are repeated here only to call attention to a theory of heredity which has obvious claims to be considered by embryologists. I have, however, looked in vain in the numerous works upon embryology and containing also remarks upon heredity, as well as most of those treating more directly with heredity itself, that have come under my eyes in the last twenty-three years, and this is certainly not an omission that tends to make such investigations more valuable to students.

TENTH LECTURE.

ON THE DEVELOPMENT OF COLOR IN MOTHS AND BUTTERFLIES.

ALFRED GOLDSBOROUGH MAYER.

If one examines the wing enclosed within the wing-case of a young lepidopterous pupa, it will be found to be transparent and of a glassy clearness. A few days before the insect is destined to emerge, however, the wings become pure white. This white soon deepens into a dull yellow or ochre color, and then the mature colors begin to make their appearance. They are faint at first, but gradually deepen into the color of the mature insect. They first appear in places near the center of the wings, upon the interspaces between the nervures, not upon the nervures themselves. Indeed, the nervures are the last places to acquire the mature coloration.

If we wish to comprehend the meaning of these color changes which affect the pupal wing, we must first study the development of the wing from its condition in the young larva up to the time when the insect is about to emerge from the pupa. In the caterpillar the future wings consist of small infolded hypodermal pockets, which are situated upon the sides of the second and third thoracic segments. The walls of these little bag-like pockets are composed of closely crowded spindle-shaped cells, and their inner cavities are penetrated by tracheæ. When the larva changes into a pupa, the wings expand to about sixty times their former area, and, as a consequence, the cells which compose the wing-bag, being no longer crowded, flatten out into a pavement epithelium. The inside of each wing-bag is hollow and contains tracheæ, blood cells, and fat cells. At first all of the cells composing the wing-bag are similar, each to each, but soon we notice that at regular intervals some of the cells are becoming larger than the others and are beginning to send out each one a process, which extends outward beyond the general level of the wing surface. These cells are destined to form the scales. The process which arises from each scale-forming cell becomes larger and flattens out into a spatula shape which exactly resembles that of the future scale. Then the cell secretes a layer of chitin over its outer surface, and thus the scales are formed. The scales are now completely filled with protoplasm and are as transparent as glass ; but soon the protoplasm begins to shrink away, leaving them little hollow, chitinous bags, which diffract the light and are therefore white in color. Soon after the scales have become hollow the blood or hæmolymph of the pupa enters them, and they become dull yellow in color ; and then the mature color begins to appear, and gradually deepens until it acquires the tint of the imago. The pigment, then, is formed within the scales during the time when they are filled with hæmolymph, and the natural inference is that the color is derived from the hæmolymph, or from some of its constituents, by unknown chemical processes. It is interesting to notice that Friedmann ('99) finds that in Vanessa urticæ the scale-forming cells, and hypodermal cells, and also the blood cells within the hæmolymph, are filled with small fatty granules which stain deeply in Hermann's fluid. These fatty granules enter the scales and contribute toward the formation of the color. No such granules were observed by me in 1896, when studying the scales of Danais plexippus and other forms ; but all of my material was killed in Perenyi's fluid or in corrosive sublimate, and Friedmann observes that corrosive sublimate fails to demonstrate the fatty globules. It is probable, therefore, that my observations are wrong in this respect.

If it be true that the colors of the mature wing are derived, by various chemical processes, from the hæmolymph, or from some of its constituents, then one ought to be able to manufacture various colors from the hæmolymph by means of chemical reagents. Also if the color so manufactured be similar to some color upon the mature wing, it may be expected to present reactions to chemical reagents similar to those of the color on the wing. As far as my experiments go I find this to be the case. For example, if one treat the hæmolymph of Samia cecropia with warm concentrated HNO_3, it congeals into a deep chrome-yellow mass. If, now, ammonia be added to excess, it changes to a reddish-orange, which is very similar in color to the reddish-orange band that crosses the upper surface of the hind wings of the moth. Now this reddish-orange band of the moth is changed to chrome-yellow by HCl or HNO_3, and then if ammonia be added, the original color reappears. Exactly the same sequence of reactions is produced with the reddish-orange pigment derived from the hæmolymph. A number of other tests of this nature were tried, and all gave confirmatory results. The details of these tests may be found in the author's (**'96**) paper on the development of wing-scales and their color.

In 1864 Landois observed that when the blood of insects is allowed to dry in the air it is very apt to assume a color which is similar to the ground-color of the wings of the mature insect from which the blood is drawn. It is well known that the most universal colors of the more lowly organized moths are the drab-gray and yellow-drab tints, and these colors may be derived from their hæmolymphs by mere exposure to the air. The brilliant reds, yellows, etc., are the result of more or less complex chemical processes, which have been slowly effected, probably through the agency of natural selection. It is interesting to notice that Hopkins finds that the white pigment found in the scales of Pieridæ is uric acid, and that the yellow and red pigments of the same butterflies are due to the derivatives of uric acid. Also Griffiths (**'92**) has shown that the green pigment of several species of butterflies is due to a complex derivative of uric acid. Indeed, the hæmolymph of the pupa is itself very complex, and it seems probable that various substances, some of them highly colored, might be derived from it by chemical processes. In the Saturnidæ, for example, the hæmolymph consists of egg-albumen, globulin, fibrin, xanthophyll, orthophosphoric acid, iron, potassium, and sodium.

When we consider the remarkable complexity of form and diversity of color found in the scales of Lepidoptera, it appears that some very potent factor must have operated to maintain them so universally upon the wings of these insects. In the first place, it is interesting to know whether the presence of scales upon the wing serves to aid the insect in its flight. In the act of flying it is obvious that the wing has two chief functions to perform, *i.e.*, it must beat downward against the air, and in the performance of this action a relatively high coefficient of friction between the air and the wing may be of advantage. But in addition to this, the wing must glide through the air, and in doing this a small coefficient of friction is desirable. There must, therefore, be an optimum coefficient of friction which lies somewhere between these two. I tested the coefficient of friction between the wing and the air in the case of several species of moths and butterflies, the wings being mounted upon a short, light pendulum, and the damping of its oscillations carefully observed. By this method it was found that the coefficient of friction of the wings remained unaltered, even when all the scales were removed from the wing membranes. We may therefore conclude that the presence of the scales does not aid the insects in flight, and it therefore seems probable that the wings of the scaleless ancestors of the Lepidoptera had already attained to the optimum coefficient of friction before the scales appeared, and that this coefficient remained unaltered during the time when the scales spread over the wing membranes.

As the scales do not aid the insects in flight, it appears that they must have been maintained and developed because their presence granted some other advantage to the insects, probably mainly because they displayed colors which may have served to aid the insects in their struggle for existence.

If one examine the colors of the wings of Lepidoptera by means of Maxwell's color disks, it is found that nearly all of the colors are quite impure; that is to say, they contain a considerable percentage of black. For example, the white ground-color of the wings of Peiris rapæ consists of sixty per cent white, seventeen per cent black, thirteen per cent emerald green, and ten per cent lemon yellow. Furthermore, a spectroscopic examination of the colors shows that they are generally compound, *i.e.*, composed of several distinct colors. It appears, then, that natural selection has not been very severe with color in Lepidoptera, for the colors are neither very pure nor intense.

When we examine the color patterns displayed by the wings of butterflies and moths, it appears that the spots show a strong tendency to be bilaterally symmetrical, both as regards form and color; and the axis of symmetry for each spot is a line passing through the center of the interspace in which the spot is found parallel to the trend of the nervures. A good illustration of this law is afforded by the case of "eye spots," where the center of the spot is always at the center of the interspace in which the spot is found. This was pointed out by Bateson in 1894. In addition to this law we notice that spots of similar form and color tend to form a row by appearing in homologous places in a series of adjacent interspaces. A study of the color patterns shows that they follow these simple laws quite rigidly, and that there is surprisingly little diversity among them. Natural selection has not been severe with color patterns in these respects, and as a consequence many patterns which we could easily picture to ourselves are never seen in nature. For example, a very striking color effect might be produced were a series of adjacent interspaces to display alternate colors, say red, yellow, red, yellow, red, yellow, etc.; but this is never seen in nature.

Considering the color patterns from another point of view, however, we cannot fail to be struck with wonder and admiration at the remarkably close resemblance between the wings of many moths and their surroundings. And the reason for this appears to be that such resemblance affords protection to the insect possessing it, and that natural selection has perfected it until now we find moths whose outspread wings almost exactly match the colors of the bark of the tree upon which they rest, etc. Even more remarkable are the numerous cases where an edible species resembles almost exactly the form and color of an immune species, which may belong to a different order or family. Bates, in his classic paper upon the "Heliconidæ of the Amazon Valley," first called attention to this remarkable fact and designated it very aptly as "mimicry." A still more remarkable mimicry is seen where one immune species resembles another immune species. For many years the explanation of this curious form of mimicry remained a puzzle to naturalists, until Fritz Müller in 1878 showed that, if it be true that young insectivorous birds have to find out by experiment what insects are good to eat and what ones are not, and if they remember to avoid evil-tasting species after once having tried them, then it becomes of advantage to both species of butterflies if one distasteful insect comes to resemble another. But we have not time to discuss this interesting topic. Suffice it to say that a series of careful and elaborate experiments, carried out by Mr. Frank Finn upon insectivorous birds of India, demonstrate that birds *do* remember to avoid retasting a noxious insect after once having had experience with it, and that most birds soon learn to beware of brightly colored butterflies which have a disagreeable taste.

In many species of moths and butterflies the males are quite different in color from the females of the same species. Darwin sought to explain this upon the hypothesis that the females exercised selection in choosing their mates, only males of a certain shade of color being popular, and that thus the males came to depart more or less widely from the females in coloration. With this hypothesis in view I have very recently carried out a series of experiments upon our common Callosamia promethea. In this moth the female is ferruginous, while the male is black. As is well known, the males of this species display a remarkable aptitude in seeking out the females, and they always fly up against the wind to the place where the female is resting. My experiments show that the males are not at all attracted by the mere sight of the female, but that they are attracted by some substance which emanates from the abdomen of the female. Females about six hours old are not as attractive as are females thirty hours old, and dead or dying females are not visited by males. Also, if the female be deprived of her eggs, she no longer attracts males to her. A male will readily mate with the females when his eyes are blinded by a covering of black glue or pitch. The male will also fly toward the females with normal eagerness even when his entire abdomen is cut off. If his antennæ be covered with balsam, glue, pitch, or flour paste, he no longer seeks the females and will neither mate with them nor display any excitement when brought into their immediate presence. If the glue or flour paste be dissolved off, however, in water, he immediately flies towards the females, as would a normal male. The males will readily mate with a female upon which male wings have been glued so that she presents the appearance of a male. They will also mate with normal eagerness with a female deprived of wings and having all the scales removed from her body. The males will fly to a piece of raw cotton within which a female is hidden, and will display intense excitement and grasp the cotton eagerly with their abdominal claspers. Conversely, the females will readily favor a male upon whom female wings have been glued so that he presents the external appearance of a female. Also they display no objection to a male who has been deprived of wings and has had all of the scales removed from his body.

As far as this moth is concerned, we are apparently justified in concluding that the male is merely positively chemotactic toward the female, being attracted by some substance which emanates from her. He is not attracted by the sight of the female, nor does the female care anything for the appearance of her consort. In this case, therefore, Darwin's hypothesis of sexual selection is not supported, and we must attempt to explain the melanic appearance of the male moth on other grounds.

In this connection it is interesting to notice that F. Plateau maintains that insects are attracted far more by the odor of flowers than by their color.

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ELEVENTH LECTURE.

THE PHYSIOLOGY OF SECRETION.

A. MATHEWS.

When a substance contained in protoplasm leaves it, it is said to be secreted, or separated from it. As all forms of protoplasm are constantly giving off matter of one kind or another, all cells are true secreting cells. Such secretion is an attribute of all living matter.

The word "secretion," as it is ordinarily used, however, is made to designate something more than this simple process of separation; it is intended to include also the process of manufacture by the cell of the substances thrown off. It is unfortunate that the same name has been given to two such different processes as those of secretion and manufacture, and it is not surprising that this has resulted in great confusion. In the present paper we shall deal solely with the separation of substances from the cell. I shall use the word "secretion" to mean this and nothing more, unless, as will be specially stated, it be used to cover the discharge from the gland duct as well. The process of the manufacture of substances by the cell I have elsewhere proposed to call "hylogenesis," literally meaning the formation of substance. This process will not be specially considered here.

How do the substances formed by protoplasm escape from it? This is accomplished in a variety of ways, depending upon whether the substances are of small molecular size and soluble, or whether they are complex bodies. Soluble substances of small molecular size escape from the cell by osmosis. Such is the case, for example, for all gases, such as hydrogen, sulphureted hydrogen, carbonic anhydride, and oxygen; for urea, nearly all inorganic compounds, the so-called internal secretions of organs of the body, such as sugar, hippuric acid, ammonium lactate, and all the simpler products of the cell's activity. Substances of this kind leave the protoplasm because their tension in the protoplasm is higher than their tension in the surrounding medium. Such substances are secreted by all cells. Since they are of such small molecular size, they readily pass from the cell as rapidly as they are formed. They never, under ordinary circumstances, accumulate in the cell. We never find any great accumulation of urea, carbonic anhydride, the internal secretions of such bodies as the supra-renals, or free sugar in the cells themselves, unless the tension of these substances in the surrounding medium is also high. Osmosis is hence the commonest mechanism of secretion. It is found in all cells of all kinds.

But besides these substances of small molecular size, the cell also produces substances of a much more complex kind, such, for example, as the albumins, mucin, glycogen, starch, proteids, fats, etc. These substances are either altogether or almost incapable of osmosis. Special mechanisms must hence be devised to eject such substances from the cells, if it be desirable that they be ejected. To accomplish this end, special devices have been developed, differing in different cells. Cells which possess these special mechanisms are those generally designated as "secreting" or "glandular-cells."

The commonest method to eliminate such substances is to break them up first into soluble and osmosable substances. They then escape from the cell by simple osmosis. This is the method by which starch and glycogen are removed. They are first converted into a simple sugar. Probably the stores of albuminous substances laid up in the fleshy cotyledons of the leguminous plants are removed in the same way, and transported in the form of these simple fragments to tissues needing them. Observation shows that during germination large quantities of the fragments of proteid molecules are set free, such as asparagin, leucin, arginin, etc. In the case of muscle the complex muscle substance appears to be broken down to such bodies as carbonic anhydride and ammonium lactate. Just how the fats escape from such cells as those of adipose tissue is unknown, but from analogy with the process of absorption of fats, it is not impossible that they are first saponified or broken into soluble glycerines and soaps. Even the ferments appear to be formed in the same manner. They exist normally in the cell in a much less soluble form, and are apparently broken up into active ferment fragments only at the time of secretion. Besides these cases in which the fragments are small enough to be removed by osmosis, there is hardly a doubt that in all gland cells this process of splitting up complex molecules into simpler ones precedes secretion, even if the process does not normally go far enough to produce an osmosable product. This process of decomposition increases the number of soluble molecules in the cell, and increases thereby its osmotic equivalent. The cell, in consequence, grows by the absorption of water and takes on the swollen appearance of many so-called loaded gland cells. This incidental result of increased cellular turgor is utilized, as we shall see, in certain processes of secretion.

The second manner in which these complex substances may be eliminated from the cell is by the budding off of a portion of the cell containing them. Actinophrys not infrequently detaches small portions of its protoplasm, which crawl about for a time but ultimately go to pieces. This is a true process of secretion. The excretophores of leeches, according to Graf, undergo a similar process. The cells loaded with secretory products approach the funnels of the nephridia and portions of them gradually break off, disintegrate, and the débris is swept out of the body. We might call this secretion by budding. Secretion here passes by a simple gradation into cell division, the sole difference being that in such secretion the bud does not contain a nucleus, and, in consequence, the budded off portion soon goes to the ground. But even this is not a hard-and-fast line of demarcation, because in many instances of cell division the cells budded off, even though containing a nucleus, undergo degeneration and are converted into a so-called secretory product. Such cases occur, for example, in the mucous glands of the skin of Petromyzon, in which the cells thus budded off are transformed into a curious mucin and bodily extruded; this happens also in the sebaceous glands, where they are converted into sebum; in the oil gland in the tails of birds; in the skin itself, in which the outer cells are converted into keratin. In fact, nearly all the appendages of the skin, such as hairs, feathers, and nails, are secretions of this sort. It will be seen that it is impossible to draw a line between such simple processes as the budding off of a portion of the cell and the well-characterized cell division. As a general rule, however, only those processes of this kind are properly designated as secretions in which the cells budded off, or given off, do not divide again, but are soon transformed into materials plainly lifeless.

A third way in which these complex substances are removed from the cell is by a compression of the cell by contractile tissue. The substances are here squeezed out and the cell temporarily disrupted. This is a fairly common method. One of the most striking and elaborate examples is furnished by the nematocysts of the Hydrozoa. In this case the hylogen to be secreted takes the form of a spirally wound thread forming a dart. This secretion is expelled violently from the cell by the contraction of a specific part of the cell's protoplasm around the cyst containing the dart. Here we have a secretion used for defensive and offensive purposes. Other clear examples of secretions of this sort are furnished by the large unicellular glands of the edge of the mantel of Aplysia and the unicellular glands of the carp-louse, Argulus. These are emptied of their watery secretion by means of a special muscular sheath surrounding the cells in Aplysia, and apparently by the body musculature in Argulus. Many of the secretions from the multicellular glands are produced in the same way. This is the case in the livers of the mollusks and arthropods, the skin glands of Amphibia, the poison glands of spiders, the sweat glands of mammals, and, as will be pointed out later, the salivary glands of mammals and cephalopods. The muscular movements of the intestinal wall are not improbably a considerable factor in most animals in the secretion of the glands and cells lining this tract.

A fourth method largely employed for removing these substances from the cell is by bursting the cell through internal cell tension. We have seen that the hydrolytic splittings taking place in protoplasm, particularly during vaso-constriction in the absence of oxygen, render the cell contents more soluble and of smaller molecular size. The absorption of water by the cell and its turgor is thereby greatly increased. A typical picture of such a cell is afforded by any mucous cell, particularly those of the submaxillary gland. The whole cell is enormously distended, and the nucleus, reduced to a granular mass, is driven against the bottom of the cell. Now, if the cell membrane be resistant enough to hold in these substances and prevent their escape by filtration, obviously there must come a time when the cell will burst. When this happens, the cell contents rush forth often with such violence that they drag the nucleus with them. More frequently, however, the nucleus remains behind and builds up a new cell. This is the manner in which the mucin appears to escape from the goblet cells of the intestine; from the intestinal epithelial cells of Ptycoptera larvæ; from the cells of the submaxillary and sublingual glands. Probably the secretion from the pancreas cells is of a similar kind, though this is by no means so certain.

Finally there is the method employed by the cells of the mammary glands in which the inner ends of the cells are disorganized and break off, apparently losing all coherence after their fatty transformation. This process is also seen in the calcareous glands of the earthworm.

In these different ways, then, do these non-osmosable substances escape from the cell. Each of these methods constitutes a mechanism of secretion, and it is readily seen that there are many mechanisms. It is hence absurd to speak of a special secretory activity of cells, as Heidenhain and many other physiologists do, as if there was one process common to all. To which of these secretory processes does such a term have reference? We shall come back to this in treating of the question of the existence of secretory nerves.

The secreting cell does not, as a rule, secrete equally well in all parts. It discharges always in one given direction. How is this brought about? It is clearly due to the fact that the cell membrane is more pervious or less resistant in one part than another. This is a matter that must necessarily be properly arranged, or the cell would discharge first in one direction, then in another. In nearly all glands the cells are so arranged that the weakest spot is toward the gland lumen and away from the basement membrane. In many cases, however, nearly the whole surface except that lying against the basement membrane permits the escape of the secreted material. This is doubtless the explanation of such pictures as one often sees in Golgi preparations of the pancreas and salivary glands, in which the impregnation of the so-called "secretory capillaries" extends between the cells nearly to the basement membrane. It is probably owing to this differentiation of the cell and the uneven resistance of the limiting membrane that cells will secrete even osmosable substances in one direction only. Consider, for example, the soluble bile salts of the liver cells which leave the cells always by the biliary capillaries. Probably where the bile capillary enters the cell is the point of least resistance, whither the bile salts must journey as fast as they are formed. The cell leaks, as it were, at this spot.

Nothing is more obvious than that the processes of secretion are under some sort of a control. Many cells and glands do not secrete constantly, but only when needed. They secrete intermittently. How is this intermittence brought about? How is secretion controlled? We shall find it necessary to distinguish in the case of multicellular glands between the discharge of secretion from the gland duct and its discharge from the cell. It by no means follows that when the cell is secreting, the secretion is escaping from the duct; nor, on the other hand, that when the gland is secreting, the cells of the gland are secreting also. The two processes often coexist, but this is by no means invariably, one might say even generally, the case. A great deal of confusion unfortunately existing in our conceptions of secretion arises from a failure to take account of this fact. In a great many cases, perhaps the majority of cases, the secretion from the cells is continuous, while the discharge from the duct is intermittent. In other well-recognized instances, however, the discharge from the cell is also intermittent.

Let us consider the simpler secretions first. The secretion of carbonic anhydride is evidently controlled by its production, since the gas is normally secreted as fast as it is produced. The production is largely dependent on the food and external conditions. In the case of muscle the secretion of carbonic anhydride is largely dependent on the contraction of muscle, large amounts of this gas being formed when muscle contracts. In this case it is under the direct control of nerves. But let it be remarked that these nerves do not control the *secretion;* they only control the production. They have no action on any hypothetical secretory function of the muscle cell at all. They cannot, hence, be called secretory nerves in the proper sense. They act on a very special substance in a very special cell. These simpler secretions, then, so far as they are intermittent at all, are rendered so by the intermittence of their production. This in turn is largely affected by many different circumstances, chief among which is the food supply of the cell. This is quite beyond the cell's control, and any nerve affecting the secretion of these substances, beyond the very special case of muscle, affects it through controlling the food supply. This is, for example, the case in the liver. Whenever the oxygen supply of the liver is reduced either by vaso-constriction, insufficient ventilation of the lungs, or in any other way, the production of sugar by the liver cell is increased and its secretion increased. Just as in the muscle, where nerves control the secretion of carbonic anhydride by controlling the production, so in the liver the secretion of sugar is controlled by its production. And if we may speak of the muscle nerves as secretory nerves, so must we equally well speak of nerves which indirectly control the secretion of sugar as secretory nerves. The vasoconstrictor nerves going to the liver or to the blood vessels of the intestinal area become, hence, secretory nerves. But they do not directly affect any secretory power of the cell; they only affect the production of sugar. They do this, moreover, by their action on blood vessels and not, as far as is known, by their action on the liver cell. All vasomotor nerves, in fact, as they control the food supply, including here oxygen, control also the rapidity of formation and secretion of certain substances of small molecular size. They become, hence, true controllers of secretion. They are secretory nerves in one sense. The secretion of all simple osmosable substances is controlled, then, by their production, which is in its turn modified by the food and oxygen supply.

How is the secretion of the more complex substances controlled? One of the most clearly defined ways in which such secretions from the cells themselves are rendered intermittent is by the action of muscle. The cells, after accumulating in themselves the hylogens to be secreted, are periodically compressed by the action of contractile matter, either within the cell itself, or immediately about it. A fine example of this sort is the nematocyst of the Hydrozoa. The hylogen elaborated lies coiled in the distended cell. When the projecting irritable point of the cell is properly disturbed, the contractile substance, lying about the cyst in the same cell, suddenly contracts and violently ejects the dart. It is not improbable that the cell then forms another dart, which in its turn when fully ready is ejected. If a secretory nerve controlled this secretion, which is probably not the case, it will be seen that it must effect the expulsion of the dart by acting on contractile tissue. It would not produce its action by altering the elaboration of the dart. A similar muscular mechanism is to be found in the unicellular glands of Aplysia, in which the enormously distended cells are surrounded by a well-marked contractile sheath. That these cells are emptied by the contraction of this sheath with a possible coincident dilation of a restraining muscular sphincter can hardly be doubted. If this secretion is under nerve control, and it probably is, as nerves have been traced to the muscular sheath, the nerves which control it must act on the contractile substance about the cell, not upon the cell itself. The large unicellular glands of the carp-louse, Argulus, are also emptied intermittently either by the contraction of the cells themselves or the surrounding musculature.

A second means by which such cellular secretions are rendered intermittent is by the periodic explosions of the cells.

In such cells, for example, as those lining the gut of Ptycoptera larvæ, a sort of mucin is formed. This absorbs water, distends the inner end of the cell until finally the turgor becomes so great that the cell actually explodes. As the nucleus remains behind, the cell is reformed, again breaks down into mucin, again explodes, and so on. The cause in this case is perfectly constant, the result only is intermittent. The secretion of the goblet cells of the intestine is probably rendered intermittent in this manner, although in this case the variation in blood supply hastens or delays the final explosion.

Finally the secretion of the cells of the vertebrate glands is controlled beyond doubt, in part at least, by the blood supply. In the vertebrate we have a highly elaborated, carefully coördinated, closed vascular system which enables an organism to turn food, oxygen and liquid, on or off from a given area at will. The cells may be thrown at once into an anæmic state or overwhelmed with oxygen. In all glands of vertebrates which are strikingly intermittent, the glands during rest are almost white; during secretion, pink, from the dilated blood vessels. That these secretions are controlled by the blood system the following facts will show: in the kidney and liver the rapidity of secretion rises and falls with blood pressure and the rapidity of flow through the glands; it is very dependent on oxygen; in the pancreas every agency which induces vaso-constriction stops secretion; every agency which induces dilation in the normal gland causes secretion; in the intestine and stomach similar relations prevail; in all vertebrate glands the principal secretory nerves are dilator nerves, the inhibitory, constrictors. Evidently, in the vertebrate, one of the chief ways of controlling secretion is through the vascular system.

Let us now consider the manner in which the discharge of the secretion from the gland is brought about. This is the process which is incorrectly called secretion by most authors. We will call it glandular secretion in distinction from cellular secretion. Let us consider the invertebrate glands first. Practically the only manner in which the glandular secretions of the invertebrates are rendered intermittent is through muscle. This muscle acts in either of two ways; first, as a sphincter around the duct, holding the secretion in so that it accumulates under high pressure. By the dilation of the sphincter the secretion pushed from behind rushes forth. Or, second, the gland or its duct is provided with a muscular sheath, and the secretion is driven out by the contraction of the sheath. In both these cases the secretion from the cell is constant; it is only the discharge from the ducts which is intermittent. In the second method mentioned above, two modifications exist; the muscular duct may be dilated into a bladder, or the muscular sheath may be around the gland.

Among the secretions which are rendered intermittent by the dilation of a sphincter may be mentioned the secretion of silk in the Lepidoptera. In this case three chitinous jaws press the thread, holding it firm; the thread accumulates under pressure in the rear. When the jaws are drawn apart by muscle the thread rushes forth. Other instances of intermittence produced by sphincters are the segmental organs of leeches, in which the vesicle is emptied by pressure, after dilation of a sphincter; the odoriferous glands of the Forficulidæ; the poison glands of insects.

The second method in which a constant secretion accumulates in a bladder with muscular walls provided with a sphincter is also common among invertebrates. Examples of such secretion are exhibited by the green glands of lobsters, the salivary glands of certain mollusks, the salivary glands of bees. Among the cases in which the gland itself is supplied by a muscular tunic, by the contractions of which it is emptied, may be mentioned the salivary glands of cephalopods, the poison glands of spiders, the pericardial glands of lamellibranchs, the livers of all Crustacea and mollusks. It may be pointed out that the nerves going to the musculature in all these cases will control the discharge from the gland and become in this sense secretory nerves.

In the vertebrates the cases in which muscle controls the intermittence are also numerous and unmistakable. Let us consider first the simplest and most obvious case — the kidney. The secretion of the kidney cells, the real secretion of the kidney, is continuous. It is, however, directly under the control of the vascular system. The secretion from the duct of the kidney is intermittent. This intermittence is brought about, as every one knows, by the interposition of a muscular bladder, or reservoir, and a muscular sphincter. The intermittence of flow from the duct is brought about, not at all by an intermittence of true secretion, but, just as in many invertebrates, by the action of a bladder. Now in the kidney no secretory nerves have ever been found; that is, no nerves acting on the kidney cells and thus controlling secretion. Nerves which control secretion, however, are undoubtedly there. Those controlling the true secretion from the cells are the vasomotors of the kidney. Those controlling the intermittence of secretion from the gland duct are the nerves governing the bladder and its sphincter. These are the secretory nerves of the kidney. Place the kidney in the bladder and the sphincter close to the pelvis, and secretory nerves to the kidney cells would have been found without difficulty. They have not been found because the relations are too plain.

The next simplest secretion is that of the liver. Here again the relationships are perfectly clear, and here again is the striking coincidence that no secretory nerves can be found. The secretion of the cells of the liver, like that of the kidney, is continuous, the secretion accumulating in the gall bladder or finding its way to the blood. Here, also, as in the kidney, the secretion of the cells is closely dependent on the blood flow. Not only is this the case in regard to the sugar secretion, as already mentioned, but the rapidity of bile secretion rises and falls, while other factors remain constant, with the blood flow. The secretion issues from the duct intermittently. This intermittence is produced by the action of the gall bladder and its sphincter, just as it is produced in the kidney and various invertebrate glands. Where are the nerves controlling this secretion? Those which control the intermittence of the gland's secretion act on the musculature of the bladder, the gall ducts, and the sphincter; those which control the true secretion of the cells act on the blood vessels either of the liver or the intestinal area.

In the stomach we have a slightly different condition. In this case the gland is inside the bladder. The relationships are no less clear. The secretion of the cells into the cavity of the stomach or the bladder is inconstant. The intermittence is controlled by the blood supply. As long as the stomach is secreting slowly, the blood supply is almost cut off the glands; when the blood vessels dilate, the glands secrete. The flow from the gland duct, in this case the duodenum, is still more intermittent. It is rendered intermittent by the action of the pyloric sphincter and the muscular stomach wall. The secretory nerves controlling the ejection of the stomach's secretion are the nerves governing the stomach's movements; those governing secretion proper are the vasomotors. The conditions are quite the same in the intestine, which may be regarded as a gland on a large scale. We have here a constant secretion rendered intermittent from the duct by the anal sphincter and the musculature of the intestine.

In the pancreas we come upon a somewhat simplified case, since here the muscular elements appear to be lacking, or, at least, of small importance. In the rabbit and other rodents the discharge from the duct and cells is continuous. In the higher mammals, for example the dog, the secretion is intermittent. The intermittence is produced by the intermittence in blood supply. The resting pancreas is very white, the blood is almost wholly cut off from it; the secreting pancreas is exceedingly red; it has a profuse blood supply. Any agency which causes vaso-dilation, provided that the gland be unpoisoned, causes secretion. For example, the gland may be caused to secrete by cutting the spinal cord, leading to vaso-dilation; by cutting the vagus, allowing it to degenerate four days and then stimulating it, a proceeding necessary to give it a dilative effect; by the drugs pilocarpine, chloral, or other vaso-dilators. On the other hand, secretion is stopped by any interruption of this blood supply; by constricting the arteries; by clamping the aorta; by any drug which causes vaso-constriction, such as digitalis or strychnine. The secretory nerves of the pancreas are the nerves governing the blood vessels. The evidence that there are any others to the gland cells we shall shortly examine.

Let us now take up one of the skin glands — the amphibian skin glands. Some of these glands may be watched living under the microscope. They are simple glands with an incomplete contractile sheath lying between the cells and the basement membrane. The glands are provided with a sphincter. If these glands be watched during secretion, it may be seen that on stimulation of the sciatic nerve (for the glands of the web) the sphincter dilates, the gland membrane contracts, and a secretion ensues. After ejecting the secretion from the gland the cells slowly secrete again, the glands become large and are under high pressure, not sufficient, however, to force the sphincter. The gland, in other words, is inside the bladder. It is exactly a case of a small stomach. The secretion from the cells appears to be constant, but it is doubtless dependent, like all other vertebrate secretions, upon the blood supply; the intermittence of the ejection is caused here, as in many other glands, with but few exceptions, *i.e.*, the pancreas, by the dilation of the sphincter and the compression of the gland. In the glands of the nictitating membrane the sphincter and the musculature appear to be innervated by nerve fibers of different origin. Drasch found that he could produce a secretion either by stimulation of the trigeminal, which caused a compression of the gland's body by contraction, or by stimulating a sympathetic spinal nerve, which dilated the orifice. Drasch himself believed that the latter nerve acted on the gland cells, because these became higher during stimulation; but it is obvious that if the sphincter opened, as he states it did, the diminution of pressure inside the gland would undoubtedly tend to increase the height of the cell and diminish slightly the diameter of the gland. Here we have, at any rate, an intermittent secretion from the ducts produced, not by intermittent secretion of the cells, but by muscle. The secretory nerves to this gland innervate the sphincter and muscular sheath. It will be noticed that here the relations are somewhat less clear and more difficult to understand, and here we begin to have evidence, though still of a very dubious kind, of the existence of nerves going to the gland cells.

The mammalian sweat glands offer the next step in the series. These are homologous with the amphibian skin glands.

Like the latter, the sweat glands are provided with a musculature between the cells and the basement membrane. There is no evidence of the existence of a sphincter. The intermittence of secretion of the sweat glands is produced in two ways. The easiest perceived is that produced by the vascular system. Dilate the blood vessels of the skin by any means, and sweat secretion, under normal conditions, always follows. It makes no difference how the dilation is brought about; we may produce it by cutting off the circulation from an area entirely for fifteen to twenty minutes. On readmitting the blood, vaso-dilation ensues, and profuse sweating. Cut the cervical sympathetic nerve in the neck of the horse, and vaso-dilation, accompanied by heavy perspiration, results. Here, as in all other vertebrate glands, then, other factors being equal and the gland in a normal state, vaso-dilation invariably causes secretion. It causes a discharge from the duct as well as from the cells, because there is no bladder and no sphincter.

There is another way, however, in which sweat secretion is rendered intermittent. This is by the actions of the musculature surrounding the gland. This drives out the fluid in the gland and its duct. This is the secretion which takes place on stimulation of the sciatic, which can go on after death, in the absence of blood supply or during vaso-constriction. If the sciatic nerve of the cat be stimulated, and during stimulation the skin plunged into some powerful and rapid fixative, the muscular sheath is found in strong contraction. This secretion generally coexists with vaso-constriction, since the same agencies which cause contraction of the sheath of the gland cause also contraction of the arterioles. Nerves which act on this sheath will be secretory nerves. Such sweat secretions are caused also by constricting drugs, such as strychnine, picrotoxine, physostigmine, and so forth. The secretion of sweat is controlled, then, in the same two ways as are nearly all other vertebrate intermittent secretions—by the action of the nerves on the muscular sheath and by the action of nerves on the blood vessels.

Another interesting skin secretion is that of the mammary glands. Here we have a constant secretion by the cells, at least nearly constant in some cases. So far as it is intermittent, it is controlled by the vascular system. After parturition the glands are gorged with blood, and secretion from the cells begins. The intermittence of the discharge of the secretion is brought about by muscles, just as it is in the other secretions examined. In this case it is largely the action of a sphincter, though doubtless the muscle of the ducts and about the alveoli comes into play. The cells secrete constantly against the sphincter, until, like the frog-skin glands or the poison glands of spiders, a high pressure is generated. The gland is put on the stretch. This pressure may even become so great that the sphincter can no longer restrain the milk which issues from the duct. The intermittence is caused by the dilation of the sphincters and the contraction of the muscle around the alveoli. In the mammary glands, also, evidence of the existence of nerves acting on the gland cells is lacking.

We come now to the most interesting glands of all — the vertebrate salivary glands. They are the most interesting, because they are the most complex, the most obscure, and possibly on account of their obscurity they have furnished nearly the whole of the evidence for the existence of special secretory nerves acting on the gland cells themselves. How are these glands rendered intermittent? In the light of the comparative study we have been through I have no hesitation in saying that they are rendered intermittent in just the same two ways as are all the other glands, *i.e.*, by the action of the vasomotors and by the action of muscle. It is another case of the gland being inside the bladder.

That the submaxillary gland is regulated in its secretion by the blood flow, not the slightest doubt can exist. The resting gland, like the pancreas, is white; the active gland, red; the chief secretory nerve is a dilator; vaso-dilation, as long as the gland is unpoisoned, always causes secretion. Thus, if the blood be cut off for fifteen or twenty minutes and then readmitted, vaso-dilation and secretion ensue. If one extirpate the sublingual ganglion leading to dilation in that gland, spontaneous secretion begins and continues. These glands are rendered intermittent, then, by the vascular changes going on in them.

There is, however, here as elsewhere, a second mechanism — that of muscle. This contractile tissue exists, like that of the skin, sweat, lachrymal, and milk glands, between the cells and the basement membrane, but it is present in much smaller amounts. The sympathetic nerve innervates this muscle, and the secretions following stimulation of this nerve, secretions coincident with vaso-constriction, secretions caused by picrotoxine, supra-renal extract, physostigmine, are caused by this muscle.

From this short survey, necessarily very incomplete, it may clearly be seen that there is not one but many different mechanisms of secretion; that secretion proper — that is, the discharge from the cell — is generally constant; its intermittence, when it is intermittent, is caused first by contractile tissue compressing the cells, second by the action of sphincters in the case of some unicellular glands, and third by the vascular system. The intermittence of discharge of the secretion from the gland's duct is due first to sphincters, second to the muscular contractions of the gland or of its ducts (bladder); or, third, to vasomotor changes in the gland. Nerves which produce this intermittence act, therefore, upon one of these three mechanisms. They do not act on the gland cell.

But besides this indirect control of secretion, do special nerves exist which control secretion by action on the secreting cells themselves? As every one knows, it is generally believed by physiologists that such nerves do exist, and that they control secretion quite apart from any condition of the vascular system, and quite apart from muscle action. It is impossible to go into this matter here at any length, — something which I have already done elsewhere, — but we can at least consider one of the main evidences for the existence of such special secretory nerves. The theory of their existence is, I believe, quite erroneous.

According to Ludwig, the originator of the theory, these nerves did not affect any peculiar secretory power of the cell at all. They caused only a decomposition of substances in the cell, which thus affected its turgor and hence secretion. In the sense originally used by Ludwig the nerves ought not properly to have been called secretory nerves ; they correspond exactly to Heidenhain's "trophic" nerves. If we mean, when we speak of secretory nerves, secretory nerves in Ludwig's sense, as is, I believe, generally meant, then all talk of secretory nerves affecting a special secretory power of the cell is wrong. They had no direct influence whatever on any hypothetical secretory power of the cell. Heidenhain, who put the Ludwig theory on its present pedestal, adopted a new sort of nerves. Those which acted on the cells, rendering their contents soluble and thus affecting osmosis, he called "trophic" nerves ; he postulated an entirely new sort of fiber which did act on the secretory power of the cell. These were the secretory fibers. They had no influence at all on the chemical processes of the cell, but only affected the resistance to filtration of the inner end of the cell. If we use the term "secretory" nerve in Heidenhain's sense, we can speak of their affecting the secretory power of the cell. Some physiologists use the term in one sense, some in another. This last theory of Heidenhain's is one of the most extraordinary, to designate it by no harsher term, of all physiological theories. It necessitates the conclusion that each single cell of the submaxillary gland, for example, has acting on it at least four different nerve fibers, *i.e.*, the trophic of the sympathetic, the trophic of the chorda tympani, the secretory of the sympathetic, and the secretory of the chorda tympani. With such consequences the Heidenhain theory is undoubtedly false. But are we in any better position if we follow Ludwig? Suppose we relinquish the trophic fibers and adopt the customary fashion of speaking glibly of secretory fibers. How do these nerves act? Do they act, as Ludwig thought, by producing chemical changes in the cell, in which case they do not directly affect any secretory function of the cell, or do they act in the Heidenhain sense on the inner border of the cell? It is all very well to speak of secretion, secretory activity, secretory nerves. In science definite meanings must be assigned the terms used. Just which of the secretory mechanisms are to be governed by such nerves? Certainly not the secretion of milk, in which the whole end of the cell goes to pieces, nor the secretion by budding, nor the explosion of the cell by turgor. Indeed, the secretory nerves, like the epicycle, are hypothetical existences devised to explain certain facts. What are those facts? If we can explain them without assuming the existence of such nerves, the hypothesis loses its value.

The most important fact is the action of atropine. If atropine be injected into a dog, vaso-dilation will still ensue on stimulation of the chorda tympani nerve, but no secretion from the submaxillary. This shows, says Heidenhain, that vaso-dilation by itself is not able to cause secretion. This inference is, as will be seen, entirely unwarranted, since it is quite possible, and is, I believe, undoubtedly true that vaso-dilation always causes secretion in the normal gland, but not in that poisoned by atropine. Atropine might have acted on the capillary walls, preventing the exudation of lymph. That it does so act there can be hardly a doubt. It might act on the gland cells, preventing them in some way from secreting in spite of vaso-dilation. Why was the latter, the natural explanation, not adopted? Because it was assumed that there was but one mechanism of secretion. There is but one mechanism of secretion, says Heidenhain. The sympathetic, hence, causes secretion in the same manner as the chorda. But the sympathetic is not paralyzed by atropine; therefore, as the sympathetic innervates the gland cells, these have not been paralyzed. But if the gland cells have not been paralyzed, and the dilator function of the chorda is unaffected, and atropine does not act on the nerve fiber, then the drug must paralyze the nerve ends of the hypothetical secretory nerve endings. The final false inference from three erroneous, or unproven, assumptions! This one example will suffice to show the character of the evidence of the existence of separate secretory nerves. Outside of the salivary glands of mammals there is no evidence worthy of the name of the existence of such nerves. It is only in those glands in which the muscle and gland cells are most closely intermingled, and relationships the most obscure, that such evidence can be found. It is to the writer almost incredible that so far-reaching and fundamental an hypothesis should have been so widely accepted on evidence of such an extraordinary character. If we only assume that there is but one mechanism of glandular secretion, — a false assumption to be sure, but one which seems theoretically probable and hence answers the prime requisite of a good hypothesis, — if we make this erroneous assumption, then the facts of secretion become so completely obscure that we are compelled to assume secretory nerves and secretory vital activities to explain them. If, however, the fact be recognized, which has stared us in the face for years, that there are several ways in which secretions are driven from glands, several of which mechanisms may coexist in one gland, then the facts of secretion, with one or two unimportant exceptions, become plain.

In closing, let us point a moral to this physiological tale. An explanation in science, whatever it may be elsewhere, is the statement of a phenomenon in simpler known terms. It is no assistance to science, but rather a scientific misdemeanor, to propose as an explanation something far more complex and difficult to understand than the facts explained. There is, to be sure, a superficial simplicity in the assumption that there is but one secretory mechanism common to all cells. But when it becomes necessary to make new assumptions to bring the theory into accord with observed fact the theory may be safely regarded as false. It was so with the geocentric theory of the universe — new epicycles had constantly to be added to account for the planetary motions; it was so with the Weismannian theory of inheritance — units of a new order had constantly to be invented; it is equally true of the secretory nerve theory.

TWELFTH LECTURE.

REGENERATION: OLD AND NEW INTERPRETATIONS.

T. H. MORGAN.

THE great interest that was awakened in the last century in the study of regeneration was the result of the experiments of Trembley, Réaumur, Bonnet, and Spallanzani. The interest aroused through the work of these four naturalists has not decreased, although from time to time other problems have come to the front and attracted the attention of investigators. More particularly in our own time has attention been directed to problems connected with the egg and its development. But it is becoming clearer, I think, that development by means of an egg and development by means of regeneration cannot be considered as separate and different phenomena, but at bottom have many factors in common. There can be little doubt that the results in one of these fields of study will throw light on the other. It will be possible for me to consider at this time only the suggestions and hypotheses advanced in connection with the problems of regeneration. At another time I shall try to compare these interpretations with those connected with the development of the egg.

Trembley's experiments on Hydra were the starting-point for the three other naturalists. Spallanzani occupied himself mainly with collecting new facts, while his friend Bonnet, who also made many new observations of great value, seems to have been more interested in the theoretical side of the problem.

Bonnet supposed that regeneration was brought about through the development of preformed germs. These germs exist in the animal solely for the purpose of replacing lost parts, and since in some animals the same part could be replaced time after time, Bonnet assumed that on each occasion a new set of germs was awakened. He pointed out that, since some animals are more subject to injuries than others, they are supplied with as many sets of germs as the times the animal is liable to be injured during its natural life.

Bonnet seems to have been especially impressed by the fact that from the same region of Lumbriculus a head or a tail may arise according to whether that region happens to lie at the anterior or posterior end of the cut surface. For instance, if the worm is cut into two pieces, a new tail will develop from the posterior end of the anterior piece, and a new head from the anterior end of the posterior piece. If, however, the cut had been made a little further in front of or behind this level, the same result would have followed; hence it is clear that at every level a head or a tail may develop. Which of these develops is determined by the position of the region, *i.e.*, whether it lies at the exposed anterior or posterior end of a piece. Bonnet interpreted this to mean that there are throughout the worm head germs and tail germs. He saw that it is necessary to give some further explanation of why the one rather than the other kind of germ is aroused to activity, and made, therefore, a further assumption. The fluids of the body that pass forward carry nourishment for the head. When the worm is cut in two, these substances are, in the posterior piece, stopped in their forward movement by the cut surface, and accumulate at this place. They act especially on the head germ, and, nourishing it, bring about the development of the new head. Similarly, fluids to nourish the tail are assumed to flow posteriorly; hence in an anterior piece of a worm they will accumulate at its posterior cut surface, and, acting on the tail germ, there bring about the development of a new tail.

In another species of fresh-water annelid Bonnet found (1745) that when the worm was cut in two a new tail developed at the anterior end of the posterior piece, and not a head. He supposed that in this worm only tail germs are present throughout the body; hence only a tail can develop, even at the anterior end of the piece.[1]

In one passage Bonnet states that the fluids that flow towards the head are there used up, and we must infer that these head-nourishing fluids are being continually made somewhere else in the body of the worm. It may be pointed out, in passing, that this idea of Bonnet's, that the fluid passing towards the head (he seems to have had the blood in view) is a special kind of fluid laden with head-nourishing substances, is not in agreement with what we know of the function of the blood or of other fluids in the body. The tissues of the head may take out of the blood those substances in it that they use in their life processes, but the blood itself going to the head is not specialized in a particular direction, and is the same fluid that flows posteriorly in other vessels.

Bonnet advanced three ideas: preformed germs; head- and tail-nourishing stuffs; and the flow of these latter in definite directions. I shall return later to these views and consider them more fully.

The process of regeneration has been often compared to the completion of a broken crystal; just as the growth of an animal or of a plant is sometimes contrasted with the growth of a crystal in a saturated solution. Herbert Spencer, in particular, has elaborated this view. In his book on the *Principles of Biology*,[2] he says: "What must we say of the ability an organism has to re-complete itself when one of its parts has been cut off? Is it of the same order as the ability of an injured crystal to re-complete itself? In either case new matter is so deposited as to restore the original outline. And if in the case of the crystal we say that the whole aggregate exerts over its parts a force which constrains the newly integrated molecules to take a certain definite form, we seem obliged in the case of the organism to assume an analogous force." Starting here with an hypothesis that is no longer held, *viz.*, "that the whole

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[1] Bonnet does not tell us how, in this case, the germs are awakened, since tail-stimulating fluids are assumed to flow backwards. Perhaps, only tail germs being present, he did not think it necessary to apply his subsidiary hypothesis.

[2] Chapter IV, Waste and Repair. First published in 1863. I quote from the last edition, 1898.

aggregate exerts over its parts a force," etc., Spencer follows this up with the *non sequitur*, "we seem obliged, in the case of the organism, to assume an analogous force."

After showing that this property, *i.e.*, "this tendency to aggregate into specific forms," cannot reside in the chemical compounds, *because*, if it resided in "the molecules of albumen or fibrin or gelatine or other proteid," there would be nothing to account for the unlikeness of different organisms; and after showing further that it cannot reside in the morphological units or cells, *because* the same power is shown by unicellular organism, Spencer concludes that this proclivity is "possessed by certain intermediate units which we may call physiological," etc.

Striking as is the comparison between the growth of a broken crystal and the regeneration of an injured animal or plant, the emptiness and superficiality of the comparison are at once apparent on closer examination. The looseness of Spencer's argument is equally evident, and his rejection of the idea that the chemical substances composing protoplasm cannot account for the facts leaves him only his imaginary physiological units to bring about the results. The latter, after all, could only be formed by combinations of the chemical substances, and if so, why introduce into the argument unknown "units" having the property of bringing about regeneration? To my mind nothing could confuse the whole subject more surely than reasoning and arguments like those advocated by Spencer.

The recent work of Rauber on the "regeneration" of crystals gives us now a basis of fact on which to rest any comparison we may make. Rauber's results show that during the growth of a broken crystal the typical form may be assumed and the broken surfaces obliterated. In some cases the growth may be more rapid over the broken surface, since this rougher surface presents a greater area for the deposition of new molecules. When a piece is broken off, the closing in of the exposed part presents no phenomena that are in any way different from the growth of the crystal everywhere else. The position of the new molecules that are added is determined by the condition at the points to which they are applied. It is misleading to speak of the "whole aggregate exerting over its parts a force," etc.

Rauber himself speaks with much reserve in his comparison between the "regeneration of the crystal" and the regeneration of animals and plants. His results show, it seems to me, very clearly that the comparison is only a general analogy; and the moment we attempt to press the comparison further it breaks down at every point.

It will, no doubt, be admitted by every one at the present time that the form of the crystal is somehow determined by the chemical composition of the substance of which the crystal is made up, and likewise that the form of an animal is also determined by the substances of which it is composed; but the broken crystal regains its original form only when surrounded by a saturated solution containing the same substance as the crystal. New substance is then added over its entire surface as well as over the part broken off. On the other hand, the process of regeneration is entirely different in an animal or plant. The new material, if any is formed, comes from within the animal or plant. Further, an animal that is slowly starving and decreasing in size will regenerate a missing part. Again, several of the lower forms regenerate by changing over the entire piece into the typical form. Can any one suppose for a moment that such a process is comparable to the re-completion of the form of a crystal?

If further evidence is asked, attention may be drawn to those cases in which an organ, different in kind from the one cut off, is regenerated. A striking case of this sort is the regeneration of an antenna in place of an eye, or the development of a tail at the anterior end of a posterior piece of an earthworm.

It would not be difficult to multiply at length these points of difference, but what I have said will suffice, I hope, to show how little we can gain by Spencer's comparison.

Pflüger[1] has given a brief outline of his conception of the process of regeneration. He says, since there is always

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[1] Pflüger, E. Ueber den Einfluss der Schwerkraft auf die Theilung der Zellen und auf die Entwicklung des Embryos, pp. 64–67. 1883.

replaced only as much as is lost, it is clear that the new limb (of a salamander) does not arise from a preëxisting germ of a limb. The wounded surface of the limb draws food material to itself, and these new food molecules are organized into a new limb. The arranging force is a molecular force that does not work at a distance from the living substance of the stump of the limb, but acts only in so far that the force draws the food molecules into the sphere of influence of the molecules in the stump itself, bringing them to a definite place, and in this way laying down a new living layer over the cut end of the limb. The way in which this new layer is organized depends on the law of organization of the part, *i.e.*, on the chemical condition of the surface layer on which the new layer is deposited. In a word, the condition of the new layer is the necessary mathematical consequence of the condition of the older regenerating layer.

On the surface of the wound a knob of indifferent tissue arises, but long before the first layer of the new material has become fully formed (differentiated ?) it has in turn acted on the succeeding layer, and this on the next, and so on, so that all the layers appear almost at the same time; but the proximal ones are somewhat nearer the definitive form than the more distal ones.

Pflüger also points out that certain exposed surfaces can organize new material only in one direction. In other words, the organized surfaces of the body show a polarization, since one side of a surface shows peculiarities not present in the other. The direction of the polarization of the regenerating surface is the cause of the direction of the new growth. For this reason a portion of an animal cannot produce the entire animal.

Pflüger has not taken into account some of the most conspicuous facts of regeneration — facts known even at the time at which he wrote. His explanation fails completely to account for those cases where a piece of an animal, hydra, for example, changes its form into a new small hydra. Moreover, Bonnet had shown that when Lumbriculus is cut in two, only a few new segments are added at the anterior end of the posterior piece. The same thing is true for the earthworm, in which, if the piece be cut off behind the fifteenth segment, no new reproductive region is ever developed. Pflüger's view leaves the absence of this region unaccounted for.

Weismann, in his book on the *Germ Plasm* published in 1893, elaborated an hypothesis of regeneration. Weismann's central idea is not different from Bonnet's (1768). Both believe in preformed germs. The differences in their views result largely from the application of modern cell doctrines to Weismann's hypothesis. Regeneration is supposed to be brought about by latent cells containing preformed germs which exist in the chromosomes of the nucleus in the form of determinants. There are supposed to be cells of this sort in the leg of a newt, for instance, at every level and in all the parts. At each level the latent cells are slightly different, and each contains germs of such a sort that all the distal part and only the distal part is represented. This germ stuff, coming into action after a series of qualitative nuclear divisions, influences the part in which it is found. Further, since the new limb will itself regenerate if cut off, the further assumption is made that during regeneration new subsidiary germs are laid down at each level in the new limb. This is supposed to take place by a division of each germ into like parts (a quantitative division) after it has reached its proper position in the new leg.

This host of invisible germs, moving at the command of Weismann's imagination, is supposed to carry out the process of regeneration. No one can fail to see that the difficulty is only shifted into a region where fancy can have free play and a scientific, experimental test cannot be applied. At one blow the difficulties are overcome, and the array of mystical germs is summoned to explain how regeneration takes place.

Since regeneration occurs in some animals and not in others, and better in those forms, Weismann thinks, that are liable to injury, additional hypotheses are added. Weismann combats the idea that regeneration is the result of the inherited effect of injuries to the part. The Lamarckian conception cannot apply in this case, since it is not the use or disuse of a part that is in question, but its power to regenerate after injury. In this I agree with Weismann, for it is no more evident how a series of injuries in succeeding generations could finally bring about complete regeneration than that the result should follow after one injury. If after the first injury, then, there is no need of any theory of inheritance.

Weismann believes, however, that the power of regeneration is under the guidance of "Natural Selection." His argument, as far as I understand it, is this: Of all the animals injured in each generation, those that regenerate better are more likely to survive; and since in each species certain organs are more liable to injury than others, the selection will take place mainly in respect to these parts; hence they possess the power of regeneration. Other organs of the body not subject to frequent injury do not show the power of regeneration, either because, having once had it, it has been gradually lost (through panmixia), or because the process has never been acquired in these organs. It may be pointed out, in passing, that since the limb of a newt and the tail of a tadpole regenerate at every level, and regenerate the kind of limb peculiar to that particular species, we must conclude, on Weismann's hypothesis, that this power has been acquired through selection for every possible level. The demand made on our credulity is enormous.

Weismann has made other statements in his book on the *Germ Plasm*, and in a later paper ('99) entitled "Regeneration: Facts and Interpretations," that are worth examining. In the former he says: "It may, I believe, be deduced with certainty from those phenomena of regeneration with which we are acquainted that *the capacity for regeneration is not a primary quality of the organism, but that it is a phenomenon of adaptation.*" Again: "Hence there is no such thing as a general power of regeneration; in each kind of animal this power is graduated, according to the need of regeneration, in the part under consideration." "We are, therefore, led to infer that the general capacity of all parts for regeneration may have been ACQUIRED BY SELECTION[1] *in the lower and simpler forms*, and that it has gradually decreased in the course of phylogeny,

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[1] The italics are my own.

in correspondence with the increase in complexity of organization; but that it may, on the other hand, be increased by *special selective processes* in each stage of its degeneration in the case of certain parts which are physiologically important and at the same time frequently exposed to loss."

There are many points in these quotations that are, I think, open to criticism, but it would lead me too far were I to attempt to discuss them. Yet I cannot let this opportunity go by without calling attention to another point raised by Weismann in his later article. He says: "It may not have occurred to Morgan that the changes in the structure of a species may have kept pace with the changes in the conditions of its life; yet this is a presupposition of the hypotheses of natural selection, and is, indeed, its *conditio sine qua non.* Hermit crabs have certainly possessed the power of regeneration 'from the beginning'; but may they not have inherited it from their ancestors, the long-tailed forms, which possess it to this day, and have need of it for all their appendages, since all are liable to injury? And cannot, nay must not, these in their turn have inherited it from their ancestors, the sessile-eyed crustaceans, and so on, through the whole crustacean pedigree, back to the unknown annelid-like ancestors of the class? . . . It seems almost as if Morgan ascribed to me the view that the capacity for regeneration must be built up anew for each species—must be inscribed, so to speak, on a *tabula rasa;* my view, however, is that here, as in all transformations, nature started with what was already present, and by modifying it brought about adaptation to new conditions. The assumed general power of regeneration in the lowly ancestors of the crustaceans would thus gradually have adapted itself to the changes in the body and to the new conditions resulting from these changes as well as from other causes; it would have become localized and specialized. . . . As in the course of time the appendages of the different body-segments became more widely differentiated in adaptation to different functions — giving rise to antennæ, jaws, walking-legs, or swimmerets — the predisposition to regenerate in certain parts of the body slowly varied also; and thus, not indeed at the same rate, but not lagging very far behind, the adaptation of the capacity for regeneration followed the adaptation of a limb to a new function."

Weismann had, in his book on the *Germ Plasm*, committed himself to the statement that the germ plasm for regeneration is different from that which brings about the development of the egg. He believed that it was necessary to make this hypothesis to account for the appearance of the so-called "ancestral organs" that are sometimes (?) regenerated. Combining this statement with what has been quoted above, it will be clear that as a new species evolves from an older one its regenerative germ plasm must also change if the animal regenerate a part like the one lost. Hence each species must acquire the power of regenerating its own particular kind of structures. This opinion I did ascribe to Weismann, and still suppose he holds to it. I did not imply that regeneration "must be inscribed, so to speak, on a *tabula rasa*." The capacity to regenerate the parts of the old species would be present in the new one, but the new species must acquire through natural selection of favorable variations the power to regenerate the new structures that have arisen through egg variations. I have quoted at length this argument of Weismann to show where we are landed by the results of his speculation. He argues for a double process of natural selection for each species that can regenerate, and is led into this position by the assumption that seems quite necessary on the preformation hypothesis that regenerative germs exist in the egg independent of the germs of embryonic development.

I find in this whole argument only an attempt to shift the difficulties of the problem back to the unknown ancestors of present forms, just as the difficulties of other parts of the problem are also shifted back upon the unknown germs that exist preformed in the egg or in the parts that can regenerate. Weismann does not, perhaps, realize the difference between himself and those whom he somewhat scornfully calls "the younger investigators." The problems that they are trying to solve are those that Weismann also tries to answer, but "the younger investigators" base their interpretations on the assumption that when a change takes place a sufficient cause for the change is to be sought in the organ itself and in the external conditions surrounding that organ. They are not content to rest their "explanations" on "the phyletic origins" of the changes. It is not necessary to deny the theory of descent, but it is unsafe and in many cases unscientific to base "causal explanations" on an imaginary line of ancestors. It is certainly unprofitable to shift our difficulties back to these historic forms, and most unfortunate to find our "explanations" also resting on the same shadowy past.

In a masterly essay, entitled "Stoff und Form der Pflanzenorgane," Julius Sachs has considered the question of regeneration in plants, and has outlined an hypothesis to account for the phenomena. Sachs bases his view on the conception that the form of a plant is the outcome of its chemical structure; that whenever the form changes there has been an antecedent change in the material (Stoff). Sachs vigorously combats Vöchting's idea that there exists in the organism a polarization of every part, and that this polarization is a directive agent that determines the kind of regeneration that takes place. No less earnestly does Sachs protest against the metaphysical conception of many morphologists, expressed or implied, *viz.*, that for each species of organism there is a form that tends to express itself and controls the development of each part. According to Sachs there are no such formative forces in the organism, but all changes are brought about by differences in the chemical composition of the "Stoff," and this leads to the development of the form peculiar to that material. For example, the flower-buds of plants are produced, not because of some innate, mystical force that causes the plant to complete its typical form, but because some substance is made in the leaves that, flowing into the undifferentiated growing point, there acts on the material of the growing point and changes it into that sort of stuff from which a flower develops.

Applying this idea to regeneration, Sachs supposes that in the plant two substances are being produced; one of these is a leaf-forming substance, the other produces roots. When a piece of the stem is cut from a plant these two substances contained in it flow in their respective directions, and bring about the production of new leaves and new roots. Sachs made numerous experiments which showed, he thought, that the direction of the flow of these substances is determined by the action of gravity; the leaf-forming substances flowing upwards, and the root-forming downwards; the direction of the flow being thus determined by some factor outside of the plant itself. It had been shown that if twigs of the willow be planted with the distal end in the ground, new roots arise from the end in the ground, and leaves from the free end. This result follows, Sachs thinks, from the direction of flow of the root-forming and leaf-forming substances.

In considering Sachs's view, it will be well, I think, to keep apart the two ideas, that specific substances produced in the plant bring about the change, and that these substances may be transported from one part to another in definite directions. We might think of the transportation taking place in a given direction as due to some peculiarity of the substance itself, or of the tissues of the organism, or, as Sachs supposed in this case, as the result of some outside influence.

It is interesting to notice that this idea of a transportation of specific stuffs goes back to Bonnet. The latter imagined head-nourishing and tail-nourishing stuffs to flow, respectively, forwards and backwards, and, acting on the germs in those parts, determine the kind that develop. Sachs does not introduce the idea of preformed germs, and correspondingly simplifies his hypothesis.

The idea of specific substances determining the regeneration of a part is, in my opinion, one deserving of very careful consideration. We have seen that the idea first suggested itself to Bonnet when searching for an explanation of the development of a new head or tail from the same region of Lumbriculus. A head developed if the exposed part lay at the anterior end of a piece, and a tail if the exposed part lay at the posterior end. Bonnet said that something must awaken the one or the other kind of germ, since both were assumed to be present at every level. Hence the idea of two specific stuffs. It is evident, of course, that the difficulty is only shifted from the germs to the stuffs, for no such stuffs were known, much less their migration in definite directions. Nevertheless, the assumption gave a formal explanation of the phenomena.

I shall next consider, in the light of the hypothesis of transportation of specific stuffs, the results of certain experiments that bear on the question.

When an oblique piece is cut from a planarian, as indicated in Fig. 1, *A*, I find that the new head develops not from the center of the piece but far up on one side, Fig. 1, *B*; and always on that side that lay nearer to the head of the planarian. The new tail develops out of that part of the posterior side that was originally nearer to the tail of the planarian, Fig. 1, *B*, *C*. The new pharynx appears in the middle of the piece, with its long axis at first in the direction of the old long axis; but as the old part changes its form to produce the body of the new worm, the pharynx comes to lie symmetrically with respect to the new head and tail, Fig. 1, *D*.

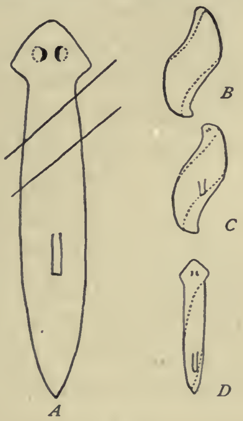

FIG. 1.

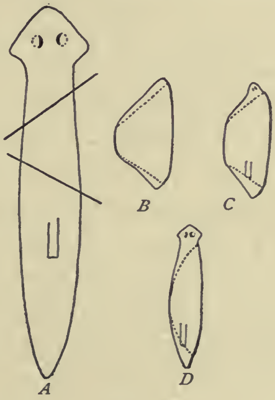

FIG. 2.

If a piece is cut from the planarian by making two oblique cuts that form an angle with each other, as shown in Fig. 2, *A*, a new head develops, as before, at the most anterior part of the anterior cut surface, and a tail at the most posterior part, Fig. 1, *B*. In this case, both head and tail lie on the same side of the piece. The new pharynx appears in the middle, but not in line at first with either the new head or tail.

These experiments show that the position of the new head and of the new tail is determined by the obliquity of the exposed part. The nearer the cut approaches a plane at right angles to the long axis, the nearer the new head and tail will be in the middle line.

If we assume that specific substances bring about the development of the new head and tail in these pieces, and also assume that the one substance flows towards the most anterior end of the piece, and the other towards the most posterior end, we should have an apparent explanation of the results.

A further application of this hypothesis may be made to certain phenomena in the regeneration of the earthworm. If the worm (*Allolobophora fœtida*) is cut posterior to the middle into two pieces, the posterior piece produces in many cases, at its anterior end, a new tail and not a head. If we assume that in the tail region only tail-forming substances are present, and little or no head-forming substance, we might offer this supposition as an explanation of the result. But a difficulty arises in connection with the idea of transportation of this stuff, for on the hypothesis it should flow backwards instead of forwards. However, the unusually long time before this sort of regeneration begins might be utilized to save the transportation theory. We might assume that the tail-forming material flowed posteriorly, but after a time so much will have accumulated that it may extend forward even to the anterior end, and there start the regeneration. It is obvious that this is a forced interpretation and that the result is not in accordance with the transportation idea.

This example of heteromorphosis in the earthworm is due, it appears, to influences within the piece itself. This seems true also for those cases, described by Herbst and myself, in which an antenna is regenerated in place of an eye in some of the crustaceans. This case also does not harmonize well with the transportation hypothesis, although, as I shall try to show later, it might be explained on the assumption of specific materials in the parts themselves.

There are other cases of heteromorphosis in which, as shown by Loeb, the influence that determines the kind of regeneration comes from the outside. In certain hydroids regeneration is influenced by the orientation of the pieces with respect to gravity; in others to light; in others to contact. In those cases in which gravity is the determining factor, we could readily imagine that the transportation of head-forming or tail-forming substances is brought about by the action of gravity. In the other cases in which light or contact has an influence on the regenerating part, it is not easy to see how a stimulus of this sort could bring about the transportation of material, although we can readily imagine that either factor acting locally might cause the production of some substance which, in turn, might act on the new tissues.

These illustrations would seem, in several cases at least, to harmonize well with the stuff-hypothesis, less well, perhaps, with the idea of its transportation. On the other hand, one does not have to look very far to find other cases to which the stuff-transportation hypothesis does not apply, or applies badly.

In the first place, in many of the lower animals regeneration does not take place by the development of new tissues, but by a remoulding — morpholaxis — of the entire piece into a new form. This side of the question has been almost entirely neglected by those who have proposed hypotheses of regeneration, and yet it seems to me that just here we find some of the most important phenomena. A protozöon cut into pieces makes as many new individuals of small size as there are nucleated pieces. The size of the new individual is, within certain limits, in proportion to the size of the fragment, and it develops — regenerates — not by forming new material at the cut surfaces, but by remoulding the entire piece into the characteristic form. If a piece is cut from a hydra, it bends together and makes a sphere or cylinder out of which a new hydra is formed. There is no evidence of the formation of new tissue, unless the tentacles are formed in that way; but in other hydroids, *viz.*, Tubularia and Parypha, even the tentacles are known to develop out of the old cells. In planarians a small amount of new tissue forms at the cut ends, and out of this a new head and a new tail develop, but the old piece changes over to form the body of the new individual. It is in the higher forms alone

— echinoderms, molluscs, annelids, and vertebrates — that we find regeneration taking place only by the addition of new tissue at the cut ends.

In those forms that regenerate by morpholaxis we must find a theory that can account for the changing over of the old piece into a new whole. To assume two formative substances might account for the changes at the two ends, but not for the rest of the piece.

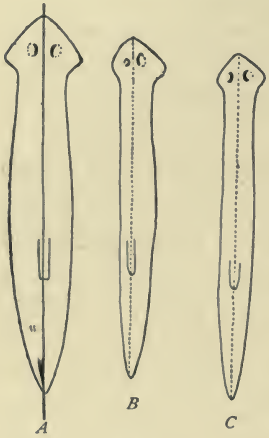

Fig. 3.

There are still other difficulties for the stuff-hypothesis. If a planarian be cut in two longitudinally, each half forms a new individual. Along the entire length of the cut edge new tissue appears. The new pharynx appears along the border between the old and the new tissue. (Fig. 3, *A*, *B*). It lies at first quite unsymmetrically in the new worm, but the new side continues to grow and the old side to get narrower. The result is that although the new worm is only as broad as the half from which it developed the pharynx lies at last in the middle plane of the body (Fig. 3, *C*).

Shall we assume that side-forming substances are present, which, being transported laterally, bring about the development of the new side? If so, we should have to assume that at each point the substance must be different from that elsewhere, for a different structure is formed. I believe that few persons would like to assume the responsibility for such a view.

One further objection. If the foot of a newt is cut off, only the foot develops; if the cut is made through the forearm, then the forearm and foot are both regenerated; if the limb is cut off through the humerus, then all distal to that point is renewed. If we assume a leg-forming substance, the assumption is insufficient to account for the differences in the result at each level. If we assume a different kind of substance flowing from the body for each level, then the hypothesis becomes extremely complicated, and we might as well fall back at once on the Bonnet-Weismann theory of preformation.

There is another experiment on planarians that has a direct bearing on Bonnet's hypothesis. If a planarian be almost entirely split in two, leaving the halves connected only at the anterior end (Fig. 4), two new heads may develop at the most anterior end of the cut edges (Fig. 4). Van Duyne, who first carried out an experiment of this sort, found two heads developing, and he interpreted their development as due to a process of heteromorphosis. I have repeated this experiment a number of times with the same results, but I think there is a simpler and more obvious way to account for the development of the new heads. They appear at the sides of each half, as they would do were a long piece cut from the side of the body; but in the latter case the result is not due to heteromorphosis. In the former case the two new heads are, after their formation, prevented from being carried forward by the presence of the old head. This interpretation is in harmony with the results of several other experiments. The bearing of this experiment on our present examination is obvious. Two new heads develop, although the old head is present. If the development of the new heads is due to the presence of head-forming substances, as Bonnet supposed, how could they develop as long as the old head is present to use up these substances? The objection might not apply with as much force if the transportation theory did not include the using up of head-forming substances in the old head.

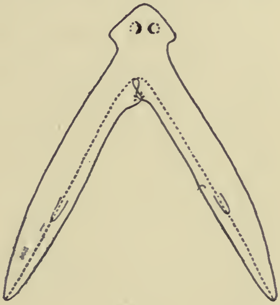

FIG. 4.

Other examples might be cited, but those given above will suffice, I think, to show the improbability of the stuff-transportation theory, or, at least, *the results show that it cannot be universal in its application* to the phenomena of regeneration. The assumption of head-forming and tail-forming stuffs is too general to explain the results. We have, however, clear evidence of the fact that the chemical composition of the various organs of the body is different, and I think it is not going too far to claim with Sachs that in a sense their form is the outcome of their composition. We have also many observations to show that during regeneration like parts form like. In the earthworm, for instance, the new ectoderm comes from the old, the new digestive tract in large part from the old one; the new nerve cord comes, in part at least, from the old one and in part from the ectoderm. In other words, the specific character of the old cells may be handed over to the new ones. If we choose to think of a formative substance in each kind of cell, we could make this the basis of our interpretation of the results; but since we do not know of such formative substances, it is safer to rest our claim simply on differences in the chemical substance of the cell itself. In other words, we only complicate our view by assuming formative substances acting on an indifferent medium — the protoplasm — and determining its changes. We need not deny that this might sometimes happen, and, in fact, several cases that Sachs has cited seem to be due to some such action; but, in general, it is not necessary to make this distinction, at least not in most cases where regeneration takes place.[1]

The assumption, in those cases in which regeneration takes

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[1] By composition of a cell or of any part of the body, we may mean either of two things. The fundamental substance may be thought of as everywhere the same, *i.e.*, indifferent, and its action is directed by grosser substances (formative substances), or we may mean by composition the entire substance without making any distinction between the substances of which it is made up. For the latter alternative we may use the terms "chemical" or "molecular" structure or "constitution," and retain the term "formative substance" for the former alternative. For example, we may think of a growing point as made up of indifferent protoplasm, and its fate determined by the kinds of formative substance carried to the growing point (Sachs). When new substance is added during regeneration at a cut surface, we may think of the new cells as at first indifferent, and their fate determined by the formative substances transported to or acting at the cut surface, or we may think of the new cells as having a molecular structure like that of the cells (or parts) from which they have come. This molecular structure might be thought of as changing later, at least so far that at different levels in the new part it changes in its relation to what is around it, and forms a new whole. The change would be due to some other factor or factors, such, for instance, as the form of the new part or its relation to the old part.

place by the development of new tissue, that the new cells have inherited their specific nature from the cells from which they have arisen, meets with no serious objection. But can we explain all the results as the outcome of this inheritance? I think not. For instance, the assumption will not explain why the new part assumes a form that is often different from that part of the body from which its cells have been derived. For example, if the first five segments be cut from the anterior end of the earthworm, five will come back; but if more than five are cut off, still only five come back. Now the new cells will in the latter instances be derived from parts of the worm that are quite different in their structure from the fifth or sixth segment, and yet from any level of the anterior end of the body only the five segments develop. The assumption of formative substances, or of a molecular structure in the new cells, might account for the fact that the new ectoderm comes from the old, the new muscles from the old ones, endoderm from endoderm, etc.; but that would leave the main problem unexplained, *viz.*, the development of a new head. This example shows also that Pflüger's hypothesis is insufficient to account for the result; for, according to his hypothesis, we should expect that at every level the whole of the missing part should be replaced, and not merely five segments, at whatever level the cut is made.

We see that other factors are also at work. Let us see if we can account for some of these. The digestive tract, at first ending blindly in front, pushes out into the new part until it comes in contact with the ectoderm. Its further growth forward is prevented, not simply by meeting a mechanical obstacle, but by what we may call, for want of a better term, a stimulus received from the point of contact. At this point the ectoderm now turns in to form, as Hescheler has shown, the buccal chamber. We might assume that the endoderm acted in turn as a stimulus on the ectoderm, and the invagination then followed; but it is probable, from certain results of Rievel and of Hazen, that the invagination would take place, even if the endoderm did not come in contact with the ectoderm. The new nervous system extends forward from the old one. In coming to the anterior end, its further growth forward is prevented by the ectodermal covering, and the cord then divides and turns upwards around the digestive tract to form the commissures and brain. Still the most essential point is unaccounted for, *viz.*, that the cord divides at the anterior end, for were the new part a tail and not a head, the division would not take place. These examples will suffice to show that the phenomena are too complicated to be accounted for by the form of the new part; however, the form of the new part may be one of the factors, but not the only one. The obvious fact that cannot be left out of account is that the new part is a head long before the cells undergo their definitive differentiation; and it seems to be owing to the new substance being from the start a head, that many of the results take place. Nothing is easier at this stage of the analysis than to drop into metaphysical ideas and speak of vital factors or formative forces, etc. I have not escaped this pitfall altogether, for I pointed out on one occasion that the process appeared as though guided by intelligence. "I mean that what we call correlation of the parts seems here to belong rather to the category of phenomena that we call intelligent than to physical or chemical processes as known in the physical sciences. The action seems, however, to be intelligent only so far as concerns the internal relations of the parts, etc."[1] The reactions to stimuli that are amongst the most common phenomena of living things is what I had in mind, and it is true that at present we cannot explain them as the result of known chemical or physical properties of matter, but I do not think that therefore I was justified in calling them intelligent processes, even in the broadest use of the word, for we thereby fall into the error of attempting to explain simpler processes by more complicated and less well-understood ones.

We are confronted, then, with the question: What is it that gives to the new part the structural character or chemical composition to form a new head? If we say its form, *i.e.*, its closed dome-shaped figure, we meet with the objection that this same form is almost universally present where the new part is present as a knob. However, since in each such knob of new cells the

[1] "Some Problems of Regeneration," *Biol. Lect.*, 1898.

cells have a different molecular structure from those derived from other regions, we might, by combining the two assumptions, escape from the dilemma. On the first assumption, that the cells of each new layer or new organ have received their molecular character from the old part, and in each kind of tissue are all more or less alike, we are driven to assume, on the second assumption, that the form of the new part determines subsequent changes in the molecular composition, and the material is so changed at each point that its arrangement produces the foundation of a new head.[1]

Before we follow further this line of thought let us examine those cases in which the entire piece is changed over into a new organism. If a piece is cut from the middle of the body of hydra, it closes in at both ends and a cylindrical form is the result. If the piece owed originally its characteristic form (*i.e.*, as a piece of the body of hydra) to its molecular structure, it would have this same character after separation; and in giving rise to a new hydra it does not develop new tissue at the two ends like that lost, but, on the contrary, after a short time the old piece itself assumes the form of a new hydra. If we hold to Sachs's view, that wherever a new form arises there has been an antecedent change in the material, we must conclude that in the piece of hydra the material has rearranged itself into a new whole. The cells do not materially change their position, but each goes over into a different part of the body from that of which it formerly made a part. Since, however, as in the case of Lumbriculus, each cell may develop into a part of either the anterior or posterior end of the new hydra, according to the level at which the piece was cut off, we must account for the one or the other change. What factors can we suppose bring about this result? In the first place, however the piece is

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[1] The specific substances that the new cells have brought with them from the old parts may in some cases determine the kind of new organ rather than the relation of the new organ to the rest of the body (through, of course, its area of contact with the old body). In this way a new eye may develop in the hermit crab when the distal end of the old eye stalk is cut off, and not an eye but an appendage when the stalk is cut off at its base. The development of a tail at the anterior end of a posterior piece of the earthworm would be due to the specific character of the new cells dominating the development.

cut out, the material at one end will have been more anterior in position in the original piece than the material at the other end. This difference is sufficient, theoretically, to account for the different results at the two ends. I do not assume that a polarization is present, because we do not know enough about any such principle, if it really exists at all, to make it of any service. My assumption rests simply on the basis that at each level the material is somewhat different from that at every other level. These considerations give us a sufficient basis to build up an idea of how the development of the piece may be thought of as taking place. As soon as the piece has been removed and its ends have closed in, it is possible to think of a rearrangement of the molecular structure taking place throughout the whole mass. Since the two ends are different, we can imagine their differences to become greater and greater in the same directions in which they differed at first. If the expression is pardonable, the anterior end becomes more anterior, and the posterior more posterior; and this influence extending to the intermediate regions, they too change in their respective directions. As a result, the material of the new piece assumes the molecular arrangement characteristic of a hydra. Then the differentiation begins that changes the entire piece into the new individual.

Let us not hesitate to push this view to its logical conclusions and ask in what part of the material does this change take place. Does each cell change and through simple contact with its neighbors bring about a change in them, and so from cell to cell? In other words, is the result an intercellular reaction? While it might be possible to look at the result as brought about in this way, still, I think there are several important reasons why we must regard the change as more fundamental than that involving only the cells as units. I cannot take up at this time my reasons for so thinking, but I may point out that since in the Protozoa and in the eggs and embryos of other forms changes similar to those I have described take place, we seem to be forced to the conclusion that the change is in the whole protoplasm and is probably a molecular change of the protoplasm.

To repeat; when a piece is cut out of the body of hydra a molecular change takes place in the protoplasm of such a sort that the entire mass is changed over into a structure that represents in its structural basis a new hydra. It is this molecular change that, dominating the subsequent development, seems to control it, and gives us the impression of formative processes at work. On my view, the formative processes are only the expression of the physical, molecular structure that has been assumed by the piece.

It will be seen at once how the same conception may be applied to those cases of regeneration in which a knob of new tissue appears and the missing part, or a portion of it, is regenerated. After the new cells have been formed they will have the same relations to each other and to the old part that the cells have in the isolated piece of hydra. The new part does not form a new whole, but only a part, simply because it is connected with the old part by the same kind of molecular union as are the cells of the new part to each other. Whether all the missing parts, as in the limb of the newt, or whether only a part, as in the head of the earthworm, will develop out of the new material, will be determined by the volume of the new material that forms, and its relation to the structural peculiarity of the new part. This sounds vague, perhaps mystical, but I think it can be given a real meaning. If, for instance, five segments are cut off from the anterior end of the earthworm, the new material that forms suffices to make five segments, but if ten be cut off, the new material is still only sufficient, owing to some molecular peculiarity, to make five.[1] In the limb of a newt we must suppose that at every level enough new material is formed at first to make possible the formation of any part of the limb.

This analysis of one of the problems of regeneration has been undertaken in order to see if it is possible at the present time to construct an hypothesis that can bring under one point of view many isolated observations. It is offered as a working

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[1] There is a difficulty, perhaps, in accounting for only two segments coming back when only two are cut off, and not five, but if it can be shown that less new material is formed the difficulty is avoided.

hypothesis or as a possible point of view, and not as an elaborated theory of regeneration. In fact, I think it would be a mistake at the present time to attempt to construct a final theory, for, if I have been in the least successful in this discussion of the problem, I hope to have made it clear that the process of regeneration involves many factors. It is obvious that until we have analyzed the problem into its component parts it would be ridiculous to attempt to formulate a theory of regeneration. It is not difficult to show that there are in reality many factors in the process quite different in kind. For instance, the closing in of the exposed surface seems to be due to some sort of cytotropism in the cells as well as to other factors; the production of new cells from the old ones must be due to another set of processes, as well as their migration out over the exposed end; the form of the piece and its size are also factors; the specific character of the cells derived from the old ones is still another problem; and finally, if my analysis is sound, the subsequent molecular arrangement taking place throughout the new part is one of the most important changes that take place. I have laid emphasis only on this latter characteristic because it seems to me that it is just this change that comes nearer to what we mean by regeneration than any of the others that I have named; they all enter into the problem, however, and none of them can be neglected, but to find a theory to account for them all at once would be, I think, extravagant to attempt and probably disastrous in its results.

THIRTEENTH LECTURE.

NUCLEAR DIVISION IN PROTOZOA.

GARY N. CALKINS.

Mitosis, or indirect division of the nucleus, with its complicated processes, is almost as characteristic of the cell as the nucleus itself. The general agreement of the separate component parts of the mitotic figure, in most cases down to the finest details of structure, makes it appear as difficult to trace its evolution as to describe the evolution of the cell itself. Yet the cell has evolved from the general to the special, and the mere fact that mitosis is strikingly similar in the most specialized of animal and plant tissues should not weigh against the view that these mitotic structures have had a history and that some stages in this history may be paralleled by structures to be found in the lowest forms of life.

All nuclei of higher plants and animals pass, during mitosis, through similar pro-, meta-, and telophases, and a similar division, or mitotic, figure is formed. But minor variations and differences in what may be homologous structures are seen in the so-called achromatic and chromatic portions of the spindle figure, differences which may be accounted for by the supposition of divergent modification of a common ancestral form. A few of the more important of these variations may be briefly considered before turning to the more generalized structures and processes in Protozoa which show, I believe, a more primitive condition. .

Broadly speaking, the variations in mitotic figures may be reduced to three types: (1) Forms with centrosome, central spindle, mantle fibers, and astral rays. (2) Forms with centrosome and astral rays but no distinct central spindle. (3) Forms without centrosome and central spindle.

In all of these cases the granular chromatin of the resting nucleus is arranged in the form of a reticulum upon a linin network, and the prophases of division are similar in that the granules are brought together and concentrated in the form of a much wound or coiled thread, — the spireme, — while the staining reactions become much more intense. This stage is universally followed by segmentation of the spireme into chromosomes of definite number, shape, and size for each species. Finally the nuclear membrane disappears and the chromosomes are left naked in the cytoplasm, but connected by spindle fibers with the two poles of the mitotic figure.

As the variations in the three types mentioned have mainly to do with the achromatic structures, the chromatin changes may be omitted. In the first type considered, the approach of division is signalized by the division of the centrosome into two daughter-centers connected by fibers which form a small spindle, called the "central spindle" by Hermann ('89).[1] This continually enlarges as the centrosomes diverge, other fibers (mantle fibers) meanwhile growing from the centrosomes and pushing in the nuclear membrane, which finally disappears, leaving the chromosomes in the cytoplasm. The mantle fibers then become attached to the chromosomes, and the latter finally surround the central spindle like a ring. The origin of these fibers is differently interpreted by different observers. The mantle fibers, according to some, arise from cytoplasmic material; according to others, from linin substance in the nucleus. The central spindle fibers, on the other hand, arise from archoplasm (Boveri), or from the substance which surrounds the centrosome (centrodesmus material of Heidenhain).

Several transitional stages between the first and the second types have been described by different observers. In these the central spindle appears first as very delicate fibers between the dividing centrosomes, but these break later and no connection remains. The complete spindle, in such a case, consists of fibers which pass, apparently, from pole to pole, with chro-

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[1] *Arch. f. mikr. Anat.*, Bd. xxxiv.

mosomes strung upon them, while central spindle fibers, if present, must be intermingled with the others, and both sets must have the same origin (Wilson, '95), *Toxopneustes*.[1] In many cases of this nature, especially in the huge mitotic figures of the first cleavage nucleus of many eggs, the spheres are relatively enormous, while the centrosomes are small and often difficult to find (*Toxopneustes* and *Arbacia*). Finally the third type of mitosis differs from the first in the absence of central spindles and centrosomes, and from the second type by the absence of centrosomes (higher plants).

While opinion differs as to the presence or absence of centrosomes in plants higher than the fungi, some observers denying, others affirming, its presence in the same species, the balance of opinion at present appears to be towards the negative side, and evidence is certainly accumulating to support this view. Nevertheless, the difficulty of proving a negative is nowhere more apparent than here, and one positive affirmation throws down the entire conception. On the other hand, positive assertions are excessively dangerous, many plastids, granules, or other products of the cell being easily mistaken for centrosomes, and only the most far-reaching experiments, with the best technical skill, can safely determine their presence. According to numerous observations by Strasburger, Mottier, Osterhout, etc., the spindle in plant mitoses arises by the gradual convergence of rays which make their appearance tangential to the nuclear membrane. Arising, as it were, from the substance of the cytoplasm, and converging to a bipolar mitotic figure, the spindle fibers are supposed, by Strasburger and his followers, to be composed of a definite and distinct substance to which he gave the name "Kinoplasm." The nuclear membrane here, as in the other types, always disappears before the nuclear plate is formed, and nuclear division proceeds in the usual way.

Leaving out of consideration for the present the discussion of the many intermediate forms of resting nuclei which, among the Protozoa, approach the type of nucleus of higher forms, we find in this primitive group so many variations of mitosis,

[1] *Journ. of Morph.*, vol. xi.

some complex, some remarkably simple, that it is possible to pick out a series which represents the stages through which the complicated mitotic figures of the higher plant and animal nuclei may have passed in their evolution to the present state. It must be distinctly understood, however, at the outset, that these variations among the Protozoa give absolutely no clue to the phylogeny of the Metazoa and the Metaphyta; all that they show, at the most, is that the various forms here considered have arrived at a certain grade of differentiation where they have stopped, although it may be logically inferred that similar stages have been passed through by the higher forms.

Beginning with the division which most closely resembles mitosis for the first type as described above, we shall see that the process in the cystoflagellate *Noctiluca* can be described in almost the same terms as that of the Metazoa.[1] The nucleus is of large size, with a distinct membrane, and with chromatin in the form of large reservoirs (*Binnenkörper* of Rhumbler, *Chromatosphere* of Doflein) from eight to eleven in number. The remainder of the nucleus is filled with large granules, which have a distinct affinity for the acid dyes. There is no trace of linin network or "achromatin" other than the large granules, which may be identical with Reinke's œdematin granules. Upon the outside of the nucleus, in the cytoplasm and close against the nuclear membrane, is a large, faintly staining mass, which corresponds with the sphere in higher cells, and which during division acts as a kinetic center.

During the early stages of mitosis the chromatin reservoirs break down, by repeated division, into an immense number of minute chromatin granules, which, at first collected in groups in the region of the reservoirs from which they were severally derived, later become distributed about the nucleus until the latter is apparently filled with them. The granules are then collected again in lines which radiate inwards from the nuclear membrane at the point where the sphere is in contact (Fig. 1, *A*); these lines form the chromosomes, which soon become double. The chromosomes thus formed are later connected

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[1] *Cf.* Calkins, Mitosis in Noctiluca miliaris. Boston, Ginn & Company, 1898.

with the centrosomes in the spheres, and then separate in the lines of previous longitudinal division. At no time is there a spireme, as in ordinary mitosis.

During early stages of nuclear activity the sphere divides into two similar halves connected by a strand composed of fibers which are formed from the substance of the sphere. These fibers compose the central spindle and are homologous in every way with the central spindle fibers of the first type of mitosis given above (Fig. 1, *A*). The nucleus then elongates in a direction at right angles to the central spindle, and at the same time it bends in the center in such a way that the central spindle sinks into a depression in the nucleus which surrounds it upon three sides (Fig. 1, *B*). In this way the nuclear plate is finally wrapped about the central spindle in the form of an incomplete ring, a condition, it will be observed, which obtains in all higher mitotic figures where the central spindle is present. The nuclear membrane then disappears in that part of the nucleus which is turned towards the central spindle, while it is retained unbroken in all other parts of the nucleus (Fig. 1, *C*, *D*). Thus the chromosomes again, as in the higher type, are brought in contact with the central spindle fibers. They then split longitudinally, and, through the agency of mantle fibers, are separated into two equal groups, each group drawn towards its own daughter-sphere. Within the sphere the mantle fibers are focused in a centrosome which at this period can be demonstrated with the greatest ease (Fig. 1, *D*). The division is finally completed by the separation of the

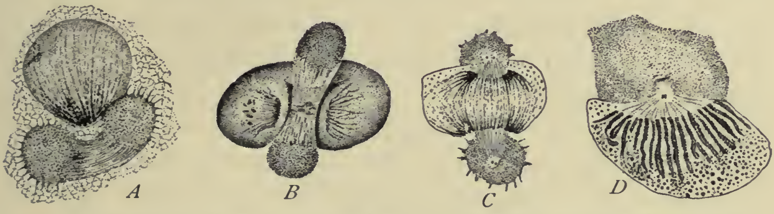

FIG. 1. — Stages in mitosis of *Noctiluca miliaris*. *A*, amphiaster and chromosome formation; *B*, metaphase showing central spindle in the hollow of the nucleus; *C*, section through long axis of the central spindle; *D*, section through one pole of late anaphase in spore-forming mitosis. Double centrosome connected with the longitudinally divided chromosomes by mantle fibers.

remainder of the nucleus and the re-formation of the daughter-nuclei, while the chromosomes disintegrate into granules which again form the large chromatin reservoirs characteristic of *Noctiluca*.

It is hardly necessary to point out further the similarity between this division figure and the mitotic figure of the first type given above. The centrosome within the sphere, the mantle fibers and their insertion in the chromosomes, the origin of the central spindle from the substance of the sphere, are features which are obviously common to the two types.

The mitotic figure, in all cases, can be easily divided into two portions, of which one consists of chromatin, the other of centrosome and spindle fibers. The two parts have more or less separate antecedent phases, and are brought in contact only during the metaphase and later stages of division. It is possible, therefore, to conceive the two processes unequally developed; a high grade of chromatin and chromosome development may accompany a relatively slight achromatic specialization, and *vice versa*. In the Protozoa, specialization of the two parts is not synchronous, and the types which are used to demonstrate the successive stages in chromosome formation cannot be used, in the same order at least, for the successive stages in achromatin differentiations. It is, therefore, more satisfactory to consider the two lines separately.

Beginning with the chromatin changes, it has been shown that in *Noctiluca* the chromatin reservoirs disintegrate into smaller and smaller pieces prior to division, and that these small parts are secondarily united into chromosomes. In many of the simpler Protozoa, notably in the Phytoflagellates, in Foraminifera (Schaudinn) and Sporozoa (Siedlecki), the chromatin material is aggregated into a single chromatin reservoir which can be compared with one of the eight or ten similar structures in *Noctiluca*. Rhumbler ('**94**)[1] described a varying number of chromatin reservoirs (*Binnenkörper*) in the foraminifer *Saccamina sphærica*, from one in the smaller forms to three hundred in the larger nuclei. *Gromia* and other reticulates have a varying number of similar masses (F. E. Schultze). In many cases

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[1] *Zeitschr. f. wiss. Zool.*, Bd. lvii.

the chromatin reservoir is known to break down into minute granules before division, as they do in *Noctiluca*. This was early made out by Buck ('**78**) for *Arcella*, by Gruber, Hertwig, and others for various rhizopods, and is therefore a widely distributed phenomenon among the lower forms.

This disintegration of the chromatin masses is a significant and suggestive process and may be regarded as universal in cell life. In the majority of cases the granules are secondarily united into chromosomes of more or less definite shape and size for each species. Among the Metazoa, Brauer's ('**93**)[1] description of the granulation of chromatin before spireme formation shows, if not identical, a closely analogous process, and the numerous observations, by various zoölogists and botanists, of spireme formation and synapsis stages, apparently belong in the same category. Among the Protozoa, Brauer ('**94**),[2] Hertwig ('**98**),[3] and Gruber ('**93**)[4] have each described the granule formation in *Actinosphærium Eichhornii*, Schaudinn ('**96**)[5] in *Actinophrys sol*, Schewiakoff ('**88**)[6] in *Euglypha alveolata*, Hertwig, Bütschli, Plate, and Balbiani in *Spirochona gemmipara*, Schaudinn, Wolters, Labbé, Clarke, and Siedlecki, in various Gregarinida and Coccidiida. Whatever may be the cause of this disintegration, possibly an expression of the "limit of growth" which Spencer postulates in connection with the relationship of surface and mass to growth, it is apparently a second stage in chromatin changes, the primary stage being the single chromatin reservoir.

The disintegrated chromatin or granules which represent the second stage in the specialization of chromatin are in reality the preliminary stage in chromosome formation; for, by their union into new aggregates, the definite bodies known as chromosomes are formed. In many Protozoa the granular stage is permanent throughout resting and active phases of the nucleus, neither the aggregation into a chromatin reservoir nor forma-

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[1] *Arch. f. mikr. Anat.*, Bd. xlii.

[2] *Zeitschr. f. wiss. Zool.*, Bd. lviii.

[3] *Abhand. d. K. Bayer. Akad. d. Wiss.*, Bd. xix.

[4] *Zeitschr. f. wiss. Zool.*, Bd. xxxviii.

[5] *Sitz.-Ber. Ak. Wiss.* Berlin, 1896.

[6] *Morph., Jahr.* 13.

tion of chromosomes taking place. Representatives of this stage are found chiefly in the Flagellata, although some Rhizopoda and some Ciliata show the same thing. Forms with this permanently granular chromatin, again, are found in two conditions. In one type the granules are scattered throughout the entire cell, and are never confined by a nuclear membrane (so-called "distributed" or "diffuse" nuclei). In nuclei of the other type the granules are confined in a definite, more or less spherical space, which may or may not be bounded by a nuclear membrane. Examples of the first type were described by Gruber in certain Rhizopoda and in a number of Ciliata, and, as he suggested, it is highly probable that many, if not all, of Hæckel's *Monera* will be found to possess nuclei of this type. Among flagellated forms it has been described by Bütschli ('**96**)[1] for *Chromatium okenii* and *Ophidomonas jenensis*, and by myself ('**98**)[2] for a species of *Tetramitus*. In the latter form the granules of chromatin, which at first are scattered throughout the entire cell with no apparent order, come together to form a loose aggregate prior to division. In this condition the aggregate is divided into halves, an equal portion going to each daughter-nucleus (Fig. 2). It is important to note here, however, that another element comes in to complicate the process. In the resting condition of the cells, when the chromatin is distributed throughout the cytoplasm, a faintly staining body can be found somewhere near the center of the cell (Fig. 2, *A*). This body becomes more definite as the chromatin granules come together for division, and it divides into two equal portions before the nucleus is halved. During the process of division the chromatin granules become heaped about this partly divided body, one-half of which remains in the center of each daughter-heap of granules until the end of the division (Fig. 2, *D*, *E*). After division the granules again separate, forming the distributed nucleus. The central body, therefore, has the attributes of an attraction sphere.

The condition represented by this temporary aggregation of chromatin granules about the sphere is permanent in the ma-

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[1] Weitere Ausführungen über den Bau der Cyanophyceen und Bacterien. Leipzig, 1896.

[2] *Ann. New York Acad. Sci.* 1898.

jority of the Flagellata, and may, perhaps, be regarded as the usual condition of protozoan nuclei. Among some Flagellata the aggregation of chromatin granules about the sphere, although permanent throughout resting and active phases, resembles the loose aggregation of the division period of *Tetramitus* in having no nuclear membrane (*Chilomonas paramœcium*, *Trachelomonas lagenella*, and *T. hispida*). In other cases, however, a nuclear membrane is present either as a faint and extremely delicate outline (*Synura*) or as a well-defined membrane (*Euglena*, *Phacus*, etc.). In the latter forms, accordingly, the nucleus has become a definite structure, consisting of chromatin granules and a kinetic center or sphere (the "nucleolus centrosome" of Keuten and other authors), and nuclear division takes place without rupture of the membrane. The chromatic and achromatic elements of the dividing nucleus are thus brought together permanently.

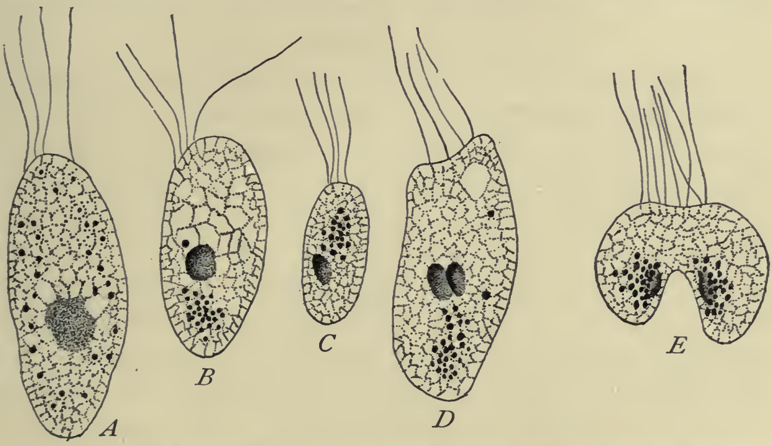

FIG. 2. — *Tetramitus* species. *A*, individual with scattered chromatin granules and central cytoplasmic sphere; *B* and *C*, the chromatin is aggregated in the vicinity of the sphere; *D* and *E*, stages in the division of the sphere and of the chromatin. Optical sections.

A third stage in the differentiation of the chromatin is seen in the secondary union of the chromatin granules to form chromosomes. In *Noctiluca*, as described, the process of chromosome formation is probably as simple as in any case where longitudinal division of the resultant chromosomes takes place. The union of the granules into larger bodies is found in many of the Flagellata with intranuclear sphere where, as for example in *Euglena*, the granules become welded together into short, thickened chromosomes, but there are no observations to show that longitudinal division takes place. A somewhat similar process of chromosome formation is described by Hertwig ('98) for *Actinosphærium*. In the latter case, as in *Noctiluca* and *Euglena*, the number of chromosomes is very great, and it is impossible to state whether the number is constant or not.

In none of these cases is there a preliminary arrangement of the chromatin into a definite spireme, as in the higher forms. In *Noctiluca* an occasional thread-like arrangement of the granules is observed, but it does not appear to be constant. In numerous other Protozoa, however, spireme formation may become as complex as in Metazoa, and, as in the latter, the thread may be segmented into chromosomes of definite shape and size, although usually of large number (*Euglypha*, *Opalina*, *Actinophrys sol*, *Acanthocystis*, *Ceratium*, etc.).

Turning now to the so-called achromatic structures of the cell, we find that the origin of the metazoan centrosome and attraction sphere from simpler elements in Protozoa has been the subject of a number of interesting theories. Most of these undertake to work out the morphogenesis of the centrosome as it appears in Metazoa. Bütschli was the first to seek its homologue in the micronucleus of Ciliata, a comparison which has found its way in nearly all of the subsequent theories. It was quickly caught up by Hertwig and by Heidenhain, and the latter, especially, elaborated it into a complicated theory of phylogeny, the main thesis being that the central spindle, as described by Hermann, is derived from the micronucleus by loss of chromatin, and becomes secondarily connected with the chromosomes of the other nucleus. Lauterborn improved the theory by assuming that micronucleus and centrosome may have had a common ancestor in some primitive binucleated protozoön, such as *Amœba binucleata* (Schaudinn), and that intermediate stages may be seen in certain existing Protozoa, such as *Paramœba Eilhardi* (Schaudinn) and *Noctiluca*. He considered the micronucleus to be a differentiation of one of these nuclei, but along a different path from that taken by the centrosome. A different theory has appeared elaborately worked out in several of the recent publications of R. Hertwig. In its latest form Hertwig's theory may be reduced to the following three stages in evolution: (1) The achromatic substance is at first uniformly distributed in the resting nucleus, but appears during division as pole plates, the equivalent of centrosomes.[1] (2) The achromatic substance becomes permanently an intranuclear centrosome. (3) It is protruded from the nucleus to form an extranuclear centrosome. The centrosome (*i.e.*, spheres) thus extruded becomes differentiated for other functions in the micronucleus of Ciliata, while the centrosome in Metazoa is defined as follows: "From a morphological standpoint the centrosomes are to be considered as portions of the achromatic substance of the nucleus which have become extruded; they are nuclear in origin. One can call them, in a certain sense, nuclei without chromatin." Thus Hertwig has introduced a new conception by regarding the centrosome as coming originally from nuclear substance. Doflein substantially follows Hertwig.

Still another point of view was suggested by Schaudinn, after his very remarkable observations and experiments upon *Acanthocystis* and *Oxyrrhis marina*.[2] He postulated two possibilities — either the centrosome is a structure formed within the nucleus, and subsequently becoming cytoplasmic, as it does in the buds of *Acanthocystis*, and as the "nucleolus-centrosome" does from the nucleus of *Oxyrrhis marina* when immersed in dilute sea water, or else the centrosome is normally cytoplasmic in position and becomes secondarily intranuclear. The latter alternative is rejected by Hertwig as far less probable than the former; his criticism being based upon his own observations and experiments with sea-urchin eggs, and upon the nuclear division of *Actinosphærium*, in which he describes the centrosome as formed from bits of chromatin. Brauer also described centrosomes in *Actinosphærium*, supposing them to

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[1] Hertwig uses the term "centrosome" in the same sense that morphologists in this country have used the term "sphere."

[2] *Verh d. deutsch. zool. Ges.*, 1896.

come from the substance of the pole plates, and hence from the nucleus, although his observations were somewhat incomplete and his account obscure. A chromatin origin of a centrosome-like body was also described by Balbiani in the nucleus of *Spirochona gemmipara*, while a nuclear origin of the centrosome among the Metazoa has been described by Brauer for the germ cells of *Ascaris megalocephala univalens*, and by Rückert for the germinal vesicle of Copepoda.

In the last two cases, however, it seems obvious that such an origin among higher metazoan cells can have little value in problems relating to the origin of centrosomes, for here differentiation is fully as complete as in any other cell, and the origin of such centrosomes can give no light on the problem. To a certain extent, Hertwig's view also is open to the same criticism, although to a far less degree. It must not be overlooked that *Actinosphærium* is a highly differentiated protozoön; its nuclei are much more like the nuclei of Metazoa than like most other Protozoa, and its reproductive processes are far advanced along the line of sexual development. It appears quite probable, therefore, that Hertwig's theory, although exceedingly ingenious and important in the light of certain facts to be mentioned later, does not go to the bottom; he picks up the thread at a point some distance from the spool, but from that point he gives a satisfactory explanation, at least from a tentative point of view, of many highly specialized processes.

Schaudinn's other alternative has opened the way for another view which explains more primitive conditions in more primitive organisms. This view, which I advanced in a previous publication, is based upon the lower flagellates and rhizopods.

It is not improbable that the condition of distributed nucleus, as in *Tetramitus*, is widely spread among the lower forms of life. Bütschli ('**96**) described it for many of the lower algæ and for Bacteria, where the nucleus corresponds closely to that of ordinary yeast cells, *Saccharomyces cerevisecæ*.[1] In *Tetrami-*

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[1] In these forms the chromatin granules lie scattered throughout the cell, or aggregated in various ways in a cytoplasmic vacuole. Like *Tetramitus*, they also have a peculiar structure (the "nucleus" of Wager) in the cytoplasm, which may

tus the sphere becomes more compact and distinct during the preparatory division stages, while the chromatin granules collect in a small aggregate in its immediate vicinity. The sphere then divides, and the chromatin aggregate separates into two portions. This stage in the division probably corresponds to the stage described by Schaudinn for the swarm spores of *Paramœba,* where the chromatin granules form a ring about the divided sphere, for, in a later stage, the chromatin granules are closely aggregated about the daughter-spheres (*cf.* Fig. 2, *E*). Here, also, Schaudinn describes the sphere (his *Nebenkörper*) as a portion of a similar body in the cytoplasm of the parent *Amœba,* from which it arises by repeated division. The swarm spores increase by binary fission, the sphere dividing first, as in *Tetramitus.* The nucleus, which is a permanent aggregate of granules, then moves around the connecting strand of the daughter-spheres, until, like the central spindle of *Noctiluca,* it is entirely surrounded (Fig. 3). The connecting strand of the daughter-spheres of *Paramœba* is homologous, therefore, with the central spindle of *Noctiluca* and of the Metazoa. After division the sphere lies upon the outside of the reconstructed nucleus, as in the resting cell of *Noctiluca.*

FIG. 3. — Development and division of the swarm spores of *Paramœba Eilhardi* (after Schaudinn).

correspond in function to the sphere, although I have not been able to follow this body in division.

It is a temporary stage like this in *Tetramitus* or *Paramœba* that lends support to Schaudinn's alternative. Intermediate stages between this condition and the permanent intranuclear sphere may be seen in numerous flagellates, such as *Chilomonas* and some species of *Trachelomonas*, where no nuclear membrane surrounds the chromatin granules. On the other hand, it cannot be denied that there is very strong evidence for Hertwig's view in the observations of Schaudinn upon the nuclei of Foraminifera,[1] and of Siedlecki upon Coccidiida.[2] In both cases the nuclei first appear as solid masses of chromatin, with (Coccidiida) or without a nuclear membrane. These become vacuolated in Foraminifera, and membrane and nuclear reticulum, as well as the definitive chromatin, are all derived from the homogeneous chromatosphere (Fig. 4). There is no mitotic figure, however, the chromatin fragmenting by what Schaudinn calls "multiple nuclear division," and the sphere, as such, does not appear in either case.

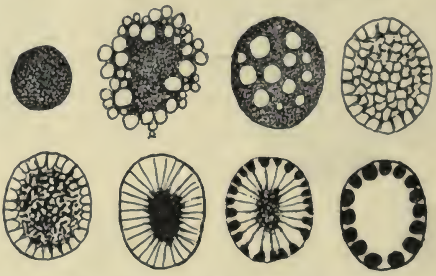

FIG. 4. — Formation of nuclear membrane and reticulum from the chromatin of an originally homogeneous chromatosphere in *Calcituba* (after Schaudinn).

Even in the lowest forms the sphere apparently exerts some force of attraction, perhaps chemotactic, upon the chromatin, and this force may or may not be strong enough to keep the granules permanently aggregated; if not, the distributed nucleus results; if so, the intranuclear condition of the sphere is the outcome. In *Paramœba*, *Noctiluca*, in diatoms, and in the majority of the Metazoa and plants, a nuclear membrane is formed, and the sphere remains outside of the nucleus. As a

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[1] *Biol. Centralbl.*, 1894, Bd. xiv.

[2] *Ann. d. l'Inst. Pasteur*, tome xii, 1898.

cytoplasmic body (archoplasm), or as a kinetic substance (kinoplasm), it may undergo further differentiation leading to the complicated mitotic figures of higher animals and plants. In all cases, however, the nuclear membrane disappears during mitosis (save perhaps in *Paramœba* and diatoms), and the original or primitive condition is thus repeated. By the disappearance of the membrane, the substance of the sphere again comes into direct contact with the chromatin.

Among the differentiations which the extranuclear sphere undergoes is the formation of spindle fibers, of centrosomes (or centrioles of Boveri and Hertwig), and of astral or cytoplasmic rays. The spindle fibers in their simplest form arise as a fibrous arrangement of the sphere or archoplasm. In *Paramœba* there is no indication of fibers; the mass of the sphere merely pulls out until the ever-thinning connecting strand is broken. In *Noctiluca* the connecting strand becomes distinctly fibrillated, the fibers consisting of a linear arrangement of the microsomes which compose the sphere. In this condition the strand may be called the central spindle, for its subsequent position in the mitotic figure shows that it has the same relations with the chromatin as the central spindle of the Metazoa, where, according to numerous observers, it arises in a similar way from the material about the centrosome (archoplasm according to Boveri, from substance of the centrodesmus according to Heidenhain). In many cases, however, the central spindle of the Metazoa is only a transient structure, the fibers of which it is composed sooner or later breaking across, so that it does not penetrate the nucleus as a spindle (many egg cells).

In plant cells, on the other hand, quite a different process seems to have taken place. Instead of a growing concentration of the sphere into a definite cell structure, there has been apparently an ever-increasing diffusion of its substance, until, as Strasburger states it, the cytoplasm is everywhere penetrated by kinoplasm, which, prior to nuclear division, comes together in lines to form the extranuclear spindle.

In those Protozoa where the sphere has become an intranuclear structure a similar history may be postulated. In some forms (simple flagellates) it retains its definite shape, although Schaudinn's observations on *Oxyrrhis marina* (a flagellate with intranuclear sphere like that of *Euglena*) indicate that the size and condition of this body are dependent upon the density of the surrounding medium. When this flagellate is placed in diluted sea water, the intranuclear body swells to many times its normal size, and may even be forced out of the nucleus, where, in the cytoplasm, it forms an abnormally large spindle; in concentrated sea water, on the other hand, it shrinks to an abnormally small intranuclear granule. This important observation opens the way for an explanation of many hitherto unexplained structures. The nucleus of *Amœba proteus,* for example, contains chromatin in the form of minute granules, which are arranged about the periphery of the nucleus, while the central portion is occupied by a large homogeneous mass, which can be explained as an enlarged or diffuse intranuclear sphere. The pole plates also, which are widely distributed throughout the Protozoa, may be explained as a temporary accumulation of this ordinarily diffuse archoplasmic substance,[1] and thus homologous with the "nucleolus-centrosome" or sphere of *Euglena* and with the centrosphere of Metazoa.

The origin of the centrosome is far more complicated, and cannot be conceived in so clear a way as the central spindle, owing to the diverse observations and the complex theories which have been made to explain it. Certain data, nevertheless, have been obtained which may serve as a basis for further considerations. The process of mitosis in *Actinosphærium* is extremely interesting in this connection, and, observed by Gruber, Hertwig, Brauer, and recently made the subject of an extensive re-investigation by Hertwig, it has become perhaps the most familiar of protozoan mitoses. Both Brauer and Hertwig describe centrosomes as transient structures in this cell, appearing, according to the former, during the anaphase of the mitosis of encysted animals; according to the latter, before and during the two maturation divisions by which the cells prepare for conjugation. Brauer's view as to the origin of the centrosome and its fate is obscure and unsatisfactory,

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[1] *Cf.* Hertwig.

while Hertwig's appears to be somewhat better founded. Both observers find that the resting nucleus consists of a basis of achromatin in the form of a network, which thickens to form a peripheral zone, while chromatin granules and plastin material lie within its meshes. When the nucleus is preparing for division, two heaps of homogeneous cytoplasm appear at opposite sides against the nuclear membrane (cytoplasmic *Kegel*). The nucleus becomes pressed together between these two masses, until it forms a lens-shape body, while within the nucleus, homogeneous masses — the pole plates — appear at each pole. Between the two pole plates the nuclear network becomes arranged in spindle fibers, upon which the chromosomes gather, at first along the entire course of each fiber, but later concentrated as distinct chromosomes in a central nuclear plate. Hertwig definitely states that the pole plates are formed from the same substance as the connecting fibers, *viz.*, the network within the nucleus, while the curious cytoplasmic and extranuclear heaps against the nuclear membrane are considered by both Hertwig and Brauer to come from within the nucleus. The substance of both pole plates and sphere, therefore, they think to be the same. In the maturation mitoses a complication is brought about by the presence of centrosomes. The formation of centrosomes is first indicated by protoplasmic radiations formed from the homogeneous extranuclear substance (Hertwig). The chromatin, meantime, collects at the pole of the nucleus turned towards this external mass, and from it a small portion is budded off to become the centrosome. This chromatin bud rapidly enlarges, assumes a spongy structure, and two granules appear within it (Hertwig calls these the "centrioles" of Boveri). After these are formed, the spongy mass (Hertwig calls it archoplasm) disappears, leaving the centrioles isolated within the cytoplasmic heap. Hertwig did not make out the origin of the other centrosome. Here, as well as in the vegetative mitoses, the nuclear membrane remains intact during the entire process; pole plates, less conspicuous than in the ordinary mitoses, are developed, and spindle fibers are formed within the nucleus.

This somewhat bizarre account of the origin of centrosomes from chromatin cannot be discarded as altogether too fanciful, for numerous observations have indicated that some similar process takes place. Thus Balbiani ('95) describes in the ciliate *Spirochona* the origin of a body which he considers to be the centrosome from the fusion of numerous granules of chromatin. Ishikawa and I had grounds for thinking that the centrosome in *Noctiluca* comes from the nucleus, while in Metazoa numerous accounts have been given of the origin of the centrosome from nucleoli and other intranuclear structures. The account of the origin of the centrosome in the buds of *Acanthocystis*, which are formed after direct division of the mother-nucleus, and which therefore contain no portion of the original centrosome, is very suggestive. Schaudinn[1] ('96) asserts that, until the fifth day after the bud is formed, there is no trace of a centrosome, but during the fifth day a centrosome appears within the nucleus and then makes its way into the cytoplasm, or rather the nucleus moves away from the centrosome and assumes an excentric position. Schaudinn does not say from what the new centrosome is formed.

While there is plenty of room for scepticism on Hertwig's observations on the origin of the minute body which he calls the centriole, the later history of this body is strikingly similar to many recorded observations upon the sphere and centrosome in Metazoa. The enlargement of the sphere and the persistence of the centriole, as recorded by Lillie, Mead, Van der Stricht, Sobotta, etc., may be described in almost similar terms. But all of these cases are different from *Actinosphærium* in that the substance of the sphere is reported as forming a central spindle between the centrosomes, and this structure, according to Hertwig, is absent in the heliozoön. What then is the significance of the intranuclear spindle fibers in this and in so many other Protozoa where pole plates are present? An answer is immediately suggested by the relation which these fibers have to the chromatin granules. Hertwig and Brauer maintain that the granules are strung upon them into chromosomes, while Schewiakoff describes a similar connection in *Euglypha*, and Schaudinn in *Actinophrys*. Central spindle

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[1] *Loc. cit.*

fibers have nothing to do with chromosomes. The intranuclear fibers of forms with pole plates thus correspond to mantle fibers of the Metazoa and *Noctiluca*, and the evidence is strong that they, like the central spindle fibers, are formed from the substance of the intranuclear sphere. The origin of the extranuclear cytoplasmic masses from the intranuclear sphere (*Actinosphærium*) lends support to the theory, first advanced by Hertwig, that the centrosome arises by emergence from the nucleus, while it also explains the origin of mantle fibers from linin substance of the nucleus as described by numerous observers upon different forms of Metazoa.

SUMMARY AND CONCLUSIONS.

The component parts of the mitotic spindle in Metazoa may be thus traced back to the generalized condition found in many Protozoa. Although many points are obscure and need verification, one fact, at least, appears well supported, *viz.*, the so-called achromatic and chromatic portions of the mitotic figure were originally more or less independent bodies which came together only during mitosis. The view advanced by Lauterborn, that the original form of the kinetic center was in some binucleated ancestor such as *Amœba binucleata*, meets with many obstacles. It must be assumed, according to this theory, that one nucleus not only changed its form, but also became completely changed in function. Again, it must be assumed that differentiation of this "kinetic" nucleus must have resulted in the well-differentiated cytoplasmic body (sphere) of *Tetramitus* or *Paramœba* as well as the intranuclear centers of many flagellates, forms which are probably as low in the scale of living things as *Amœba*. It seems hardly necessary to regard this body, in its original condition, as a nucleus which later becomes differentiated into a well-defined kinetic substance with degeneration of the original nuclear contents. It seems much more simple, and, in the light of facts, much more probable, that the kinetic substance was as definite a portion of the original primitive cell as the nucleus itself, although formed possibly from metamorphosed chromatin. This practically restates Boveri's archoplasm theory as he gave it out twelve years ago — a theory which has been virtually unaccepted so far as the structures for which it was developed are concerned, but which finds its greatest support in the Protozoa. Originally cytoplasmic, this substance, after taking the form of a definite kinetic body, became intranuclear during division of the cell — a condition which is permanent in the majority of the Protozoa, and which is repeated, during mitosis, by every cell throughout the animal and plant kingdoms.

Within the nucleus the archoplasm body, at first definite and compact (Flagellata), became secondarily diffuse, appearing indefinite in structure (*Amœba proteus*) or evenly distributed as linin throughout the nucleus (*Actinosphærium*, *Euglypha*, *Actinophrys*, and nuclei of higher animals and plants), while, during division, it now re-collects to form the pole plates (*Infusoria* and many *Sarcodina*), or spindle fibers and "archoplasm" (Metazoa).

The origin of the extranuclear sphere may be interpreted in two possible ways. It may be regarded as a direct continuation of the primitive cytoplasmic condition through forms like *Tetramitus*, *Paramœba*, and *Noctiluca*, where it never becomes intranuclear more than the central spindle does in the latter form; or it may be regarded as nuclear in origin, being secondarily extruded from the nucleus, as Hertwig describes in the case of *Actinosphærium*, or Schaudinn in the case of *Acanthocystis*. In the former case both Brauer and Hertwig are agreed that the cytoplasmic mass (*Kegel*) comes from the achromatic substance of the nucleus, hence from the intranuclear archoplasm, and as this substance becomes the matrix for the later appearing centrosomes, it is not incorrect to speak of it as the sphere.

Whatever its primitive relation to the nucleus may have been, the cytoplasmic sphere is large and permanent in *Noctiluca*, but it becomes smaller in Metazoa; and just as it becomes diffused throughout the nucleus in Protozoa and Metazoa, so it may become diffused throughout the cytoplasm in Metazoa—a condition which Boveri postulated in his remodeling of the archoplasm theory ('95). In higher plants, also, it may be regarded as permanently in this diffused condition, as Strasburger practically postulates in his kinoplasm theory. In plants, however, no centrosphere is formed, the spindle fibers alone representing the archoplasm. The increasing diffusion of the substance of the sphere (archoplasm) explains the variations in the mitotic figures as given at the outset of this chapter, the central spindle gradually becoming fainter and fainter in a series of forms, until it finally is lost. It also explains a number of anomalous cases, such as the formation of asters in poisoned echinoderm eggs, described by Hertwig, or the artificial asters recorded by Morgan.

The mantle fibers, or fibers which connect the kinetic centers and the chromosomes, also have an archoplasmic origin, and are primarily nuclear in position. In this primitive condition they are seen in *Actinosphærium* (Gruber, Hertwig, Brauer), in *Euglypha* (Schewiakoff), in *Spirochona* (Bütschli, Plate, Hertwig, Balbiani), and in micronuclei (Hertwig, Maupas). In all of these cases they run from pole to pole, or from pole plate to pole plate. With the extranuclear position of the kinetic center, the rupture of the nuclear membrane becomes necessary, for only then can the union of central spindle and mantle fibers take place. With this in mind the controversy over the nuclear or cytoplasmic origin of mantle fibers gains an added interest; for in either case they are formed from the same substance, which may be either nuclear or cytoplasmic in position.

A comparison, I believe, may be drawn between chromatin and archoplasm. In its earliest form the chromatin is massed in one or several large reservoirs, which later break down into granules distributed throughout the nucleus. The granules may be permanent or temporary, but in all cases they come together again as chromosomes. Similarly, archoplasm, in its primitive form, is a single mass which becomes diffused throughout the nucleus and cytoplasm, but in all cases comes together again in the form of spheres or spindle fibers. As chromatin is considered a definite substance or modification of protoplasm, so also may archoplasm be regarded in the same light.

The centrosome question, finally, is made no clearer by study of the Protozoa. Recent observations lead to the view that it is not a permanent organ of the cell (Brauer, Hertwig, Schaudinn, Morgan, etc.), while its complete absence in higher plants and its appearance in certain mitoses while absent in others of the same species (*Actinosphærium*) justify Flemming's view that centrosomes may be present in mitosis, but not necessary for it. The origin of the body, which in various cases is called the centrosome, is equally unsatisfactory. Hertwig's and Balbiani's view, that it comes from chromatin which may (*Actinosphærium*) or may not (*Spirochona*) become extranuclear, is opposed by the views, often hypothetical, of many observers upon centrosomes in Metazoa. Further and guarded observations, especially among the Protozoa, must be made before the various conflicting views can be reconciled.

COLUMBIA UNIVERSITY,
December 1, 1899.

FOURTEENTH LECTURE.

THE SIGNIFICANCE OF THE SPIRAL TYPE OF CLEAVAGE AND ITS RELATION TO THE PROCESS OF DIFFERENTIATION.

C. M. CHILD.

During the sixty years since the cell theory was propounded by Schleiden and Schwann (1838–39) it has come to dominate almost completely the various departments of biological research. In the introduction to his book, *The Cell* (**'97**), Wilson remarks that "it has become even more clearly apparent that the key to all ultimate biological problems must, in the last analysis, be sought in the cell." Notwithstanding the wide acceptance of the cell theory, a voice of protest has from time to time been heard, from both the botanical and the zoölogical side, based upon actual observation as well as upon theoretical considerations. Prominent among those who have upheld what Whitman (**'93**) designates as the "organism standpoint" are Sachs, Rauber, Adam Sedgwick, and Whitman himself. It is not my intention to enter into a historical discussion of the views of the various authors, but to direct attention to one phase of the problem, *viz.*, the relation of the process known as differentiation or organization of the cell.

As is universally recognized, differentiation may occur within the limits of a single cell, as in the Protozoa, or within an organism consisting of many cells, and, therefore, cannot necessarily be connected with the process of cell formation. This fact is most clearly expressed by Whitman as follows: "It is not division of labor and mutual dependence that control the union of the blastomeres. It is neither functional economy nor social instinct that binds the two halves of an egg together, but the constitutional bond of *individual organization.* It is not simple adhesion of independent cells, but integral structural cohesion.

"That organization precedes cell formation and regulates it, rather than the reverse, is a conclusion that forces itself upon us from many sides. In the Infusoria we see most complex organizations worked out within the limits of a single cell. We often see the formative forces at work, and structural features established before fission is accomplished. Cell division is here plainly the result, not the cause, of structural duplication. The multicellular Microstoma behaves essentially in the same way as the unicellular Stentor or the multinucleate Opalinopsis of Sepia. The Microstoma organization duplicates itself and fission follows. The chain of buds thus formed bears a most striking resemblance to that of Opalinopsis, and the resemblance must lie deeper in the organization than cell boundaries.

"We must look entirely behind the cellular structure for the basis of organization."

It is the continuity of organization, whether the organism be composed of one cell or many, which is insisted upon throughout this paper, and in agreement with Sachs (**'82**), cell formation is regarded as of only "secondary significance."

Wilson (**'93**, p. 595) stated in regard to Amphioxus that "*the unity of the normal embryo is not caused by mere juxtaposition of the cells. They* (the facts) *indicate that this unity is not mechanical but physiological, and point toward the conclusion that there must be a structural continuity from cell to cell that is the medium of coördination and that is broken by mechanical displacements of the blastomeres.*"[1]

McMurrich (**'95**), in a lecture of this series delivered in 1894, after a discussion of the early development of centrolecithal ova, concludes "that in embryological development the differentiation which occurs is a differentiation of the entire organism and not of the constituent parts of which it is composed; physiologically, if not morphologically, every organism is a

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[1] Italics in this and in all succeeding quotations are as in the original.

syncytium, and future theories of heredity must take this into consideration."

Even more directly, I believe, do the facts point to the existence of such a structural continuity in the eggs of annelids, mollusks, etc., where the division of a single cell at other than the proper time would in many cases disarrange the whole complex. The theory of prearranged harmony makes too great a demand upon the cell as distinguished from the organism to be within the limits of probability in this case.

We have, moreover, as a basis for our belief in the continuity of organization, not merely the results of comparative study of the unicellular and multicellular forms and the inferences from experiment, but there are a rapidly increasing number of observations showing the presence of actual protoplasmic continuity between cell and cell. Observations of this kind upon plants have been very numerous and include a wide range of forms. (For the literature upon this subject see Zimmermann, '93, and A. Meyer, '96). In animal tissue intercellular connections have been found to be of wide occurrence among epithelial cells, and from time to time, especially during the last few years, our knowledge of these structures has been increased by their discovery under many other circumstances. A brief résumé of the state of our knowledge at the time his book appeared is given by Wilson ('97), concluding as follows: "As the subject now lies, however, the facts do not, I believe, justify any general statement regarding the occurrence, origin, or physiological meaning of the protoplasmic continuity of cells; and a most important field here lies open for future investigation."

It is interesting to contrast with Wilson's somewhat conservative view the opinion expressed by A. Meyer ('96) in a paper published almost at the same time that Wilson's book appeared. After a résumé and discussion of the observations along this line upon both the botanical and the zoölogical side, Meyer sums up his views as follows: "So far as I can see at present, all our knowledge upon this subject favors the view that protoplasmic connections occur between practically *all* cells of every individual, that a characteristic of both the animal and vegetable individual is the possession of *one continuous mass of* *cytoplasm*. Whether the individual consists of a uninucleate cell, a multinucleate cell, or a system of cells, the cytoplasm forms a continuous whole."

And in another paragraph he says: "They (the protoplasmic connections) do not appear to be, like the nerve fibers, alloplasmatic organs adapted for special purposes, but strands of normal cytoplasm which connect the cytoplasm of neighboring cells. Their significance is probably to be explained as follows: All activities and material changes in that part of the multicellular system (the individual) which we designate as a protoplast have a direct effect upon the constitution of these protoplasmic processes, and the alterations which occur in the organization of these exercise, in their turn, a direct influence upon the activity of the neighboring cells."

Fig. 1. — A small part of the blastula of the starfish, showing the intercellular connections (after Andrews).

Hammar's observations upon sea-urchin eggs ('96) were made upon fixed material, but since these were made a very interesting series of observations upon living cells have appeared (G. F. Andrews, '97; A. E. Andrews, '97a, '97b, '98a, '98b, '98c), which must, I believe, lead us to the conclusion that intercellular protoplasmic continuity between animal cells is at least of very wide occurrence, if not universal, during developmental stages (Figs. 1 and 2). This being the case, we have, it seems to me, very strong evidence upon both the botanical and zoölogical side for the correctness of the so-called organism standpoint, as distinguished from any cell theory. And I believe we must admit that intercellular protoplasmic continuity is of the greatest importance in our interpretation of the organism.

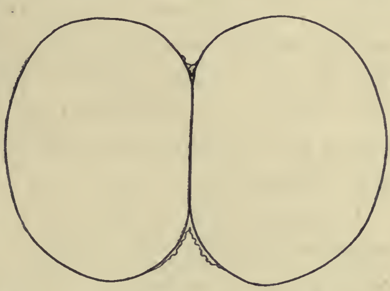

Fig. 2.—Two-cell stage of the egg of a fresh-water nemertean, showing the ectoplasmic activity in the cleavage furrow and the rounded form of the cells immediately after division.

Although my study of cleavage was begun from a point of view coinciding fully with the cell theory, my observations have led me to the belief that any explanation of the spiral cleavage is impossible upon that basis. The developing egg of the turbellarian, annelid, and mollusk is, I believe, more than a cell mosaic; it is distinctly an organism at every stage.

I desire, then, to discuss the spiral type of cleavage, not as a series of self-differentiations or cellular interactions, but as a series of processes, each of which has its cause in the organism, as distinguished from this or that cell. From this point of view alone, as I believe, is it possible to explain the various phenomena observed, and especially the lack of correspondence in origin and fate of the blastomeres, which is not explicable by any strictly cellular theory of development.

In his classical paper upon *Nereis*, Wilson ('92) has given us a most valuable discussion of the origin of the spiral form of cleavage, and it is desirable at this point to review this briefly. In general, his view is as follows: "*The fundamental forms of cleavage are primarily due to mechanical conditions, and are only* *significant morphologically in so far as they have been remodeled by processes of precocious segregation.*" As regards the spiral cleavage, he believes that it "*is a secondary derivative of the true radial type,*" being the result of "*mutual pressure* among the cells." Furthermore he says: "But the remarkable fact, and one which does not seem to be very clearly recognized, is that the effect of these mechanical conditions *has become hereditary.*" And again: "We now come to the main point, which is that *the rotation* of the cells is in the spiral type *in many cases predetermined in the parent cells, as is proved by the position of the spindles and by the form of division.*" And lastly: "To sum up, I conclude that the spiral form of cleavage is owing to a precocious appearance of the alternation of the cells, which, in its turn, is a result of mutual pressure."

The question now arises as to the significance of the mutual pressure and the reason for the establishment of the spiral cleavage as hereditary. Discussion along this line must, of course, be of a purely speculative nature, and, although there is a certain truth in Driesch's contention that explanations of this kind do not explain ('99), I still believe that they are of value as showing, perhaps, some of the steps in the process, and a possible or probable motive for these steps, even though the why and the how of the reaction of the organism is a mystery.

It is clear that the alternation of blastomeres that would result from mutual pressure, etc., produces a form of cleavage in which the blastomeres, like soap bubbles, conform to the law of minimal contact surfaces, and that therefore each blastomere is in contact with the greatest number possible of the others. From the so-called organism standpoint such a relation must unquestionably be advantageous, unless we assume that the peculiar property of protoplasm which binds the cells into an organism is capable of some mysterious "Fernwirkung," which does not seem probable. Roux's "Cytotropismus" appears, perhaps, to be such a "Fernwirkung," but I would like to suggest the possibility that this so-called cytotropism may resolve itself in some cases into the establishment of extremely fine protoplasmic connections.

From the standpoint of the cell theory this arrangement of the blastomeres would appear distinctly disadvantageous, for here a much larger portion of each blastomere is in contact with others than in the looser cell aggregate, so that respiration and excretion apparently cannot occur as freely as in a loose mass of rounded cells. The periodic appearance of the large intercellular cavities filled with liquid, as described by Kofoid ('95) for *Limax*, and which occur in other forms as well (Figs. 3 and 4), is probably a provision for aiding the excretion, some such provision having become necessary under certain conditions (fresh-water or terrestrial life in moist localities), in consequence of the extremely intimate union of the blastomeres.

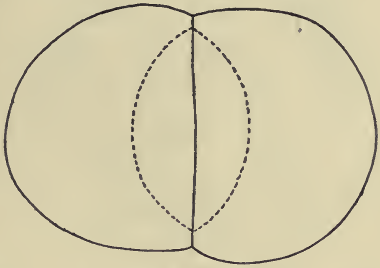

FIG. 3. — Two-cell stage of a fresh-water nemertean after the attainment of the resting stage, showing the flattening together of the cells and the temporary intercellular cavity.

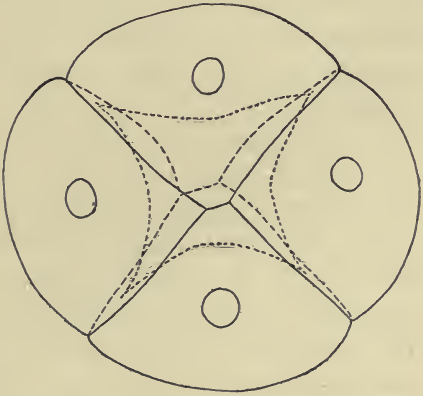

FIG. 4. — Four-cell stage of a fresh-water nemertean, showing the temporary intercellular cavity.

Moreover, we do find that in many forms, and especially in those in which the spiral type of cleavage occurs, there is a distinct condensation of the process of development, which finds expression in the early appearance of differentiation and morphogenesis for forms in which any such tendency existed; it must be clear that a conformation to the principle of minimal contact surfaces among the blastomeres will afford the most direct and intimate communication possible between them.

It is then, I believe, on account of the necessity for just this intimate communication between the component parts of the organism that the spiral cleavage, which in its simplest form fulfills these conditions in the most perfect manner possible, has become so firmly established, even in spite of the fact that the free respiratory and excretory surfaces of the cells have been reduced, in consequence of its presence, to such an extent that in some eggs a compensatory arrangement in the form of the intercellular cavities is necessary.

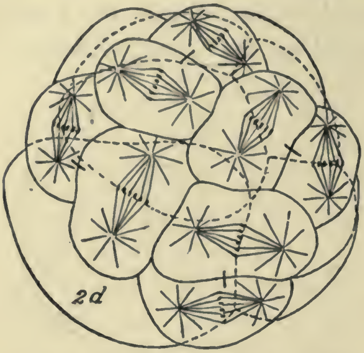

FIG. 5.—*Arenicola.* Fifth cleavage viewed from upper pole. *2d*, the "first somatoblast" already formed.

How this process has occurred we cannot explain at present, but that it *has* occurred I believe we must admit, and that there is sufficient motive for its occurrence I trust I have shown.

The next point which requires investigation is the fact that, in the great majority of cases, the direction of corresponding cleavages is the same, *i.e.*, the second cleavage is læotropic and the third dexiotropic, and so on (Fig. 5). Why should this constancy occur? Directly correlated with this problem is the question as to the cause of the few cases of reversed cleavage in gasteropods.

If we suppose the cleavage to be hereditary, the direction of the early cleavages is no longer a matter of chance, and the direction of succeeding cleavages would follow according to the principle of regular alternation. This being the case, the assumption is not at all unwarranted, that, of the two possibilities, one has become established and the other has disappeared. What factor determined which one of the two possibilities should become the rule is beyond our knowledge, but it is certainly a fact that in nearly all spiral cleavages the second division is læotropic and the third dexiotropic, etc.; and it is just as certain that this constancy is not merely the result of mutual pressures in groups of four, eight, or more blastomeres.

The occasional appearance of reversed cleavage is explicable as a variation from the normal type, — the only variation possible with continued conformation to the spiral type, — or in some cases it may be that this variation for some special reason has become the rule. According to this view the reversed spiral cleavage has arisen from the more common type.

Our knowledge of the cleavage of reversed forms is very incomplete as yet. As regards *Planorbis*, Rabl ('79) shows clearly a reversal of the usual direction of cleavage, and the work of Holmes ('97) affords confirmation (Fig. 6). Crampton ('94) discovered that the cleavage of *Physa* is reversed. The case of *Janthina* is doubtful. Haddon has figured an apparent left spiral in the third cleavage, but *Janthina* is, at least usually, dextral. Conklin ('97) points out the possible causal relation between reversed cleavage and reversed asymmetry in the adult, but the point I desire to emphasize is the probable secondary nature of this reversal, its occurrence as a variation somewhat of the nature of a sport, which, at least in some cases, was inherited and became the rule. If we accept this view, the occurrence of sinistral gasteropods must be regarded as without phylogenetic significance.

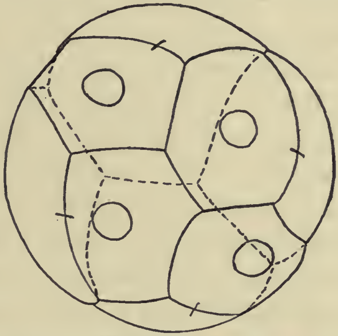

FIG. 6.—*Planorbis*. Eight-cell stage. An example of reversed cleavage (after Holmes).

We find among the gasteropods some species with occasional sinistral individuals, others in which the sinistral individuals are more common, and still others in which all are sinistral. A study of the cleavage in species where sinistral forms are common is greatly needed. I am inclined to believe, however, that reversed cleavage precedes reversed asymmetry, and further that the cause of the reversed asymmetry is the reversal of the cleavage and not *vice versa*; and, finally, that cases of reversal are secondary modifications, and have arisen after the relation between cleavage and adult asymmetry, of which *Crepidula* affords an example, had been established.

Having reached this point in our consideration of the spiral cleavage, it is pertinent to inquire whether we find in this type of cleavage, as it exists at present, any evidence of the continuity of organization, or whether we must admit, as appears to be generally believed, that the cells possess a high degree of independence.

And first we turn to the evidence furnished by experimental work upon the spiral type of cleavage. Evidence of this kind is limited to Crampton's experiments upon *Ilyanassa* (**'96**), Wilson's upon *Nereis* (**'92**), and Fujita's upon *Aplysia* (**'97**), which latter form, according to Blochmann's account of the cleavage (**'83**), adheres strictly to the spiral type.

Crampton succeeded in isolating various blastomeres of *Ilyanassa*, some of which continued to develop for a longer or shorter period. As I showed in 1897, he is in error in regarding the larvæ resulting from these isolated blastomeres as strictly partial larvæ, since, as he himself states, the entodermal blastomeres are completely enclosed by ectoderm, which would be impossible without modification of the form of cleavage. In order to accomplish this enclosure the ectoderm cells must cover a greater amount of surface than that normally covered by them, for, in addition to covering the outer side of the large entodermal blastomeres, they must also cover that portion of these cells which is normally in contact with the other entodermal blastomeres. This modification in form after the injury and the complete closure of the blastopore indicates that the mutilation has affected the whole organism and that a partial rearrangement has occurred. And even as regards the earlier cleavages of isolated blastomeres, which Crampton has studied in detail, there is evidence in his figures of some degree of modification in the form of cleavage. The fact that the earlier cleavages do continue so nearly as if the rest of the egg were present, even though the blastomeres assume a spherical form, seems to me to indicate simply that the form and direction of cleavage are so fixedly determined by heredity that the shape of the cell has little influence upon them. Intercellular protoplasmic connections would also tend to keep the form of cleavage constant. But gradually a rearrangement does occur, or complete closure of the blastopore would be impossible. The fact that the prototroch is only partial is due to the early differentiation of these cells. It is not proven, moreover, that it would not have been completed if the larvæ had lived long enough.

A very interesting result obtained by Crampton is the failure of those eggs in which the yolk lobe had been removed to form a cell corresponding to the mesoblast in structure, although a cell which is packed with yolk spherules, like the other entodermal cells, is formed. According to Crampton the yolk lobe appears to consist almost wholly, or wholly, of yolk spherules; and in any case it cannot contain the bulk of protoplasm represented by the mesoblast, so that it is apparently not the loss of mesoblast material, but some other effect of the mutilation which results in the defect. The result of this experiment indicates, it seems to me, a considerable amount of rearrangement of the egg substance.

But perhaps the most interesting result of all these experiments is the extreme sensitiveness of the egg to injury, as indicated by the very small proportion of mutilated eggs which continued to develop. This fact points most unmistakably to the existence of a very high degree of interdependence among the blastomeres. In most cases the portion of the egg observed, although itself apparently uninjured, is so crippled by the loss of the part removed that death ensues. Even in those cases where development continued for a time the embryo never succeeded in completing it, but sooner or later succumbed.

Wilson succeeded in modifying the cleavage of *Nereis* by means of pressure, so that the micromeres were not formed till the fourth division, and then eight of them appeared above eight more, larger, yolk-laden macromeres. These eggs developed into trochophores with double the usual number of entodermal blastomeres. Here again the insignificance of the cell as compared with the organism is apparent.

I have not had access to Fujita's paper upon *Aplysia*, but in a brief abstract of it, it is stated that he found that isolated blastomeres were able to restore the lost portions and to reassume the normal shape. Whether the rearrangement occurs directly or in later stages is not stated. In any case the effect of the mutilation upon the remaining portions is evident. If complete rearrangement does take place, it may be because the egg of *Aplysia* is more hardy than that of *Ilyanassa*.

Turning now to the normal cleavage, we find there a number of phenomena which, as I believe, can be satisfactorily interpreted from the organism standpoint alone.

And first I am inclined to believe that the flattening of the first two blastomeres and of later ones against each other, a phenomenon of very wide occurrence (*cf.* Figs. 2 and 3), may be the result of material connections between the cells, which bring the cells into contact with each other over the largest possible portion of their surface after the intracellular pressure accompanying mitosis has begun to diminish.

In many cases the reversal of the direction of spiral cleavages, especially when occurring in one or two quadrants, is indicative of interrelation; for, as Conklin states ('**97**, p. 186), the ultimate cause of reversal is, in most cases, the precocious appearance of certain organs or planes of symmetry.

The relative time of differentiation of various organs, and especially of the early larval organs, such as the prototroch, affords to my mind a most striking example of the interrelation of all parts of the developing egg. For instance, in *Amphitrite* and *Lepidonotus* the primary trochoblasts become ciliated before the sixty-four-cell stage (Mead, '**97**). In *Nereis* the cilia appear at about the tenth hour, at a stage when the closure of the blastopore is nearly completed. In *Arenicola* the first traces of ciliation appear when the embryo is from seventeen to nineteen hours old, after the blastopore has closed completely and the embryo consists of hundreds of cells. In *Crepidula* the trochoblasts are very small and remain apparently quiescent up to a late stage, then increasing in size, and still later attaining their definitive functional condition. In *Ischnochiton* (Heath, '**99**) the velum becomes ciliated much earlier than in *Crepidula*, — thirty-one hours, — and there is no marked increase in size of the cells. Now in all these cases the so-called primary trochoblasts arose in a very similar manner and at essentially the same time in the history of the egg, yet for some reason the time of formation appears not to be related directly to the time at which they become functional.

It is important to note that in each case the differentiation occurs at such a time that the trochoblasts shall be prepared to perform their function when called upon by the environment.

The simplest explanation would seem to be that the energy of the egg is used where most needed. In cases where the larva swims at a very early stage, the cilia appear correspondingly early, but in other cases the trochoblasts remain apparently at rest, perhaps for a long time, till some time before they are needed the differentiation appears. To me this appears to be simply a case of the saving of energy. The energy of the egg is so exactly distributed that none is wasted in the development of organs before they are needed. The distribution of the energy must be connected with some physical or chemical change, and in this way intrinsic differences may arise between the various cells, but the differences between the quiescent trochoblasts and the other cells do not necessarily signify that the former contain a special substance which makes them distinctively trochoblasts from the time of their formation. Of course at some time they do become distinctively trochoblasts, but simply because of their relation to the whole.

Again, if the course of the cleavage in different forms be followed, the different time relations in the division of the various cells indicate the nicest adjustment to prevailing conditions. I cannot see how such adaptations could arise, nor how others can arise in the future without the closest relation between the parts.

The close relation of many cells to their environment, even after differentiation, is evident from many of the facts of regeneration, and experiments on the early developmental stages of many eggs lead us to the same conclusion. In those cases which seem to point to a directly opposite conclusion, it is sometimes perfectly clear that the apparent independence of the cell or cells in question is really due to differentiation, but there is also reason to believe that in many cases the essential features of the environment are not recognized, and that what appears to be independence is in reality dependence upon factors of the environment whose existence is unsuspected.

It is difficult to understand how "precocious segregation" could have appeared at all without a very intimate interrelation of parts; but, on the other hand, it seems clear that the existence of a high degree of continuity would favor it. Once established, however, as we find it, there is little reason to suppose that, since its establishment, the blastomeres have lost their connection — physiological or otherwise — with each other, unless the existence of a high degree of self-differentiation is actually proven.

Now the spiral cleavage is, as shown above, the most favorable form of cleavage possible for the establishment of physical continuity of organization, for each cell is brought into contact with the greatest possible number of cells. Is it not a justifiable conclusion that its wide occurrence, firm establishment, and invariability, especially in those forms in which a condensation of development — a precocious segregation — is found, are due, at least in some measure, to the fact that it affords the least possible physical obstacle to intercommunication between the component elements? For, if a tendency to condensation be present, the possession or acquisition of the spiral form of cleavage must be of great advantage. Or it may be that the presence of this form of cleavage has led to that form of condensation known as precocious segregation. Whichever alter-

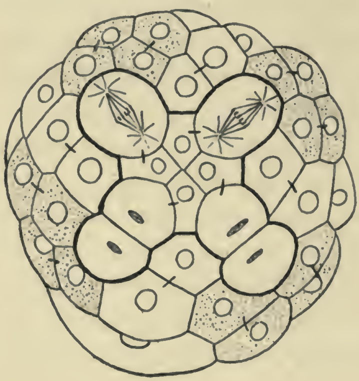

Fig. 7.

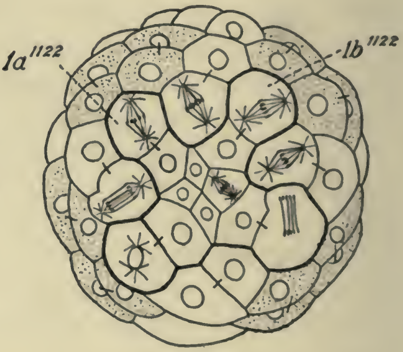

Fig. 8.

Fig. 7.[1] — *Arenicola*. Formation of cross by radial divisions.

Fig. 8. — *Arenicola*. The terminal cells of the ventral arms of the "cross" show spindles which are distinctly spiral.

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[1] In this and following figures of cleaving eggs the annelid cross ("rosette-series" of Conklin) and its descendants, and the somatic plate arising from *2d* are enclosed by a heavy line; and the cells of the prototroch and paratroch, so far as formed in each case, are stippled.

native is the correct one, it seems clear that the spiral form of cleavage and precocious segregation are very closely connected in annelids and mollusks.

In the forms under consideration — forms possessing spiral and determinate cleavage — there is imposed upon the spiral cleavage proper a strictly bilateral form of cleavage, in which the axes of symmetry are the same as in the adult (*cf.* Fig. 5 with Figs. 7 and 8, also Fig. 9 with Fig. 10). This bilateral type of cleavage is evidently connected with the process of morphogenesis, and I desire to call it, for convenience, a *morphogenetic cleavage.* A morphogenetic form of cleavage

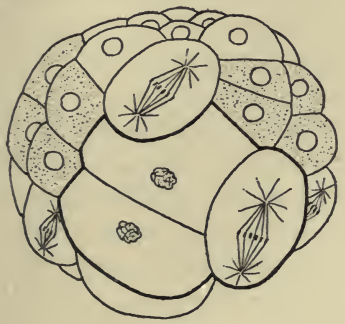

FIG. 9.

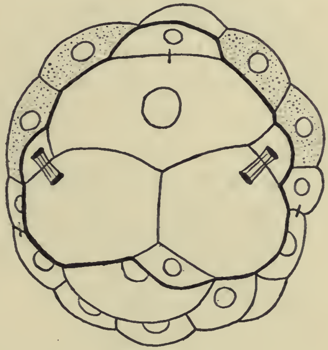

FIG. 10.

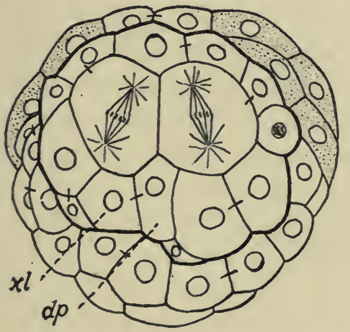

FIG. 11.

FIG. 12.

FIG. 9. — *Arenicola.* Somatic plate consists of three cells which have arisen by spiral divisions.
FIG. 10. — *Arenicola.* Somatic plate after spiral divisions have ceased.
FIG. 11. — *Arenicola.* Somatic plate. The cell *dp* gives rise to the two dorsal cells of the paratroch, the cell *xl* to the two lateral paratroch cells of the left side.
FIG. 12. — *Arenicola.* Somatic plate. The cell *dp* dividing spirally.

is not merely determinate, as Conklin has defined the term, but the various processes of cleavage are so arranged that the interaction of the component parts leads directly to the establishment of form. The factors of morphogenesis in cleavage are direction and time of division and size of the products, accompanied, of course, by more or less differentiation, according as morphogenesis proceeds more or less rapidly; but differentiation, as I believe, is a process wholly distinct from cleavage, whether cleavage be indeterminate, determinate, or morphogenetic. Morphogenetic cleavage, of course, possesses in general a real phylogenetic significance.

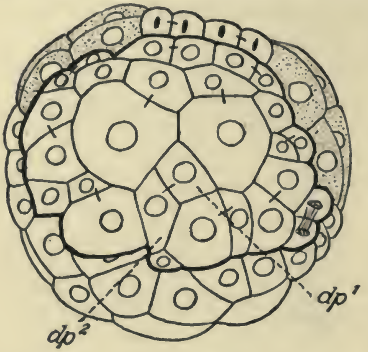

FIG. 13.—*Arenicola*. Somatic plate. dp^1 and dp^2, cells resulting from division of *dp*; at the top of cut the four "intermediate girdle cells" which are formed in front of the prototroch, but come to lie behind it.

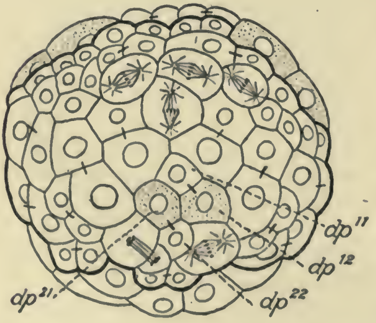

FIG. 14.—*Arenicola*. Somatic plate. Dorsal cells of paratroch formed.

A sufficient cause for the establishment of this form of cleavage is, I believe, the saving of energy which must occur. If the energy of cell division can be utilized to bring material to the point where it is needed, and at the time when it is needed, it is evident that less energy will be required than if extensive migrations were necessary after the formation of the cells. A study in detail of the growth and concrescence of the somatic plate in *Arenicola* (Figs. 9–18), or in any other form in which it has been fully worked out, affords one of the most beautiful illustrations of the perfect coördination in morphogenetic cleavage, and I think that no one who follows it can doubt that each and every cell division plays its part in bringing about the concrescence with the least expenditure of energy.

Since every cell plays a definite part, it is evident that morphogenetic cleavage is necessarily accompanied by a certain degree of segregation, *i.e.*, the material which is to form the various organs is more or less completely segregated into definite cells or groups of cells, though they may not be differentiated, or, rather, the differentiation may be simply one of position.

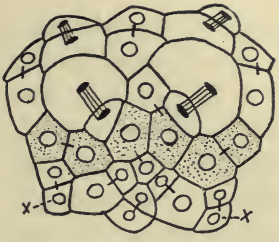

Fig. 15.—*Arenicola.* Portion of somatic plate, showing position of paratroch when first formed. *x x*, the first cells which meet in the median line during concrescence.

It appears probable that this bilaterally symmetrical, morphogenetic cleavage has encroached upon the spiral period during the course of phylogenetic development and shortened it, and that, in addition, independent modifications have arisen in the different groups, thus altering the relations of the constituent elements. A study of the differences in cleavage in various forms brings to light a number of facts which appear to justify these conclusions.

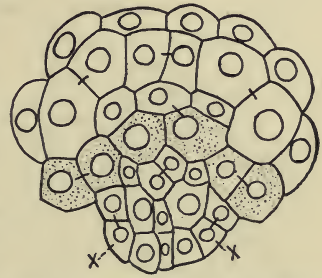

Fig. 16.—*Arenicola.* Portion of somatic plate. Concrescence proceeding.

In the following paragraphs I have brought together some of the facts which appear to me to furnish evidence upon these points.

1. The spiral period proper ends with different cell generations in different forms.

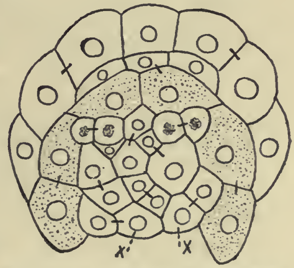

Fig. 17.—*Arenicola.* Portion of somatic plate. The cells *x x* in contact in median line.

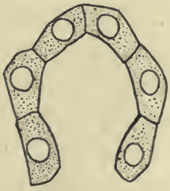

Fig. 18.—*Arenicola.* Paratroch just before ventral ends are brought into contact by the concrescence of somatic plate.

Meager as are the data for a comparison between the different larger groups, they indicate what I believe will be amply demonstrated in the future, *viz.*, that *as the process of development becomes more and more highly modified, the strictly spiral cleavage, when originally present, gives way at an increasingly early stage to a morpho-*

genetic form of cleavage, which finally attains its most pronounced expression in teloblastic growth.

A comparative study of the cell lineage of various Polychæta shows a considerable degree of difference within the limits of this group as regards the stage at which the spiral form of cleavage disappears (*cf.* Figs. 7, 19, 20), but it is impossible to discuss this point in detail here.

2. Cases of what may be called "reversion" to the spiral type of cleavage occur, *i.e.*, cells which have departed from the spiral type may return to it in later stages for one or more generations. Numerous examples of this "reversion" occur. I wish to mention here some of my own observations on *Arenicola* without reference to other forms, for, where special attention has not been paid to this point, figures and general statements are usually too indefinite to serve in this connection. The cross in *Arenicola* is formed by radially symmetrical divisions (Fig. 7). In the division following this the four cells terminating the arms of the cross often show traces of spiral divisions. This is especially noticeable in the ventral arms of the cross (Fig. 8, $1a^{1122}$, $1b^{1122}$), which often curve at the tip toward the right side of the egg. Both the spindle and the position of the cells after division indicate in many cases the spiral nature of the cleavage. The direction is læotropic, as it

FIG. 19.

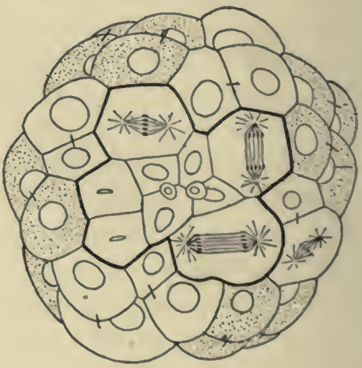

FIG. 20.

FIG. 19. — *Podarke.* Cross formed by spiral divisions (after Treadwell).

FIG. 20. — *Chætopterus.* Cross does not appear because of extreme spiral character of divisions which commonly give rise to it (after Mead).

would be if the regular alternations of the spiral period had continued.

In the formation of the two dorsal paratroch cells (Fig. 14, dp^{12}, dp^{21}), the last two divisions which they and their sister cells undergo are, so far as direction and alternation are concerned, true spiral divisions, although they give rise to a bilaterally symmetrical group of cells. The first of the two is læotropic (Fig. 12, *dp*, Fig. 13, dp^{1}, dp^{2}), the second dexiotropic (Fig. 14, dp^{11}, dp^{12}, dp^{21}, dp^{22}). The cell (Fig. 12, *dp*) from which the four are formed is a cell of the tenth generation, and if all the preceding divisions had been spiral it should divide læotropically as it does. But in the ninth generation the spindle was inclined sinistrally, though approaching horizontal (Fig. 11, *dp*, *xl*), while in the eighth and seventh generations the spindles were both inclined dextrally.

Under this head the fact may also be mentioned that in *Arenicola* some spindles, which are meridional or horizontal in later stages of mitosis, are sometimes at first distinctly spiral. One of the best examples of this kind is the first somatoblast after its first two divisions. The third cell (Fig. 10) lies, when formed, directly anterior to the large stem cell, and the spindle in later stages is perfectly meridional, but often, even up to the equatorial plate stage, it is distinctly dexiotropic, as it normally would be in spiral cleavage.

Conklin has given a table of the reversals of cleavage in *Crepidula*, of which there are a considerable number. Some of these, however, are peculiar and possess especial interest with respect to the relation of spiral and bilateral cleavage to each other. These are described as follows: "In a few cases which are classified here as reversals, the nuclear spindle does not indicate that the cleavage is to be reversed, and even the daughter-nuclei may occupy the same relative positions as in the quadrants in which there is no reversal, while at the same time the lobing of the cytoplasm and the subsequent rotation shows that the cleavage is reversed; the first division of 3*d* (Figs. 25, 26, 29) is a case in point. In such cases the conditions which influence the direction of the cleavage are not manifested until after the nuclear division is completed, whereas they are usually shown in the direction of the nuclear spindles and in the earliest stages of cleavage." These observations, which, so far as I know, have not been duplicated in the study of any other form, indicate, it seems to me, that the reversal is of later date than the original spiral and has been imposed upon the older form, but without removing all traces of it as yet. I have sought very carefully for similar phenomena in *Arenicola*, but without results except those mentioned above. Reversals occur but rarely in *Arenicola*.

3. The spiral form of cleavage ceases first in regions which are to play important rôles in the later development (Figs. 7, 10), while the regions which do not represent large areas in the later stages continue to divide spirally for some generations. In many of these cases it is impossible to say where the spiral form of division has given place to a bilateral form, for there is no sudden change, but the spiral direction simply becomes less and less marked in succeeding divisions, until it is no longer distinguishable as such. The cells of the third quartette of ectomeres in *Arenicola* afford an excellent illustration of this point. Conklin states that in *Crepidula* the bilateral divisions appear very slowly and that it is difficult to determine in some cases whether the divisions are really bilateral or not. This, I think, will be found to be the case in many forms. It is certainly true of *Arenicola* as well as of *Crepidula*. A few cells contrast sharply with the rest of the egg as regards the initiation of bilateral cleavages, and these are the cells whose divisions distribute the material for the embryo, including the other cells themselves to a large extent.

4. The probability that independent modification of the bilateral form of cleavage has appeared in the different groups or species is amply supported by the fact that in many cases cells of similar origin have different fates, and *vice versa*. It is sufficient here to mention a few of the more striking cases of this sort. Perhaps the most striking case of all is that of the annelid and molluscan cross. In each group the cells of the cross form the larger portion of the pre-trochal region, though the exact relation of the various cells to the apical plate is not sufficiently well known at present to afford a basis

for close comparison. The cross does not consist of the same cells in the two groups. As Conklin has shown, the cross of *Crepidula* arises mostly from the cells corresponding to the so-called "intermediate girdle cells" of the polychæte, which there form a relatively small portion of the pre-trochal region. On the other hand, the cross of the polychæte is composed of the cells which Conklin calls the "rosette series," and these certainly do not form as large a part of the pre-trochal region in *Crepidula* as in the polychæte (Figs. 21, 22).

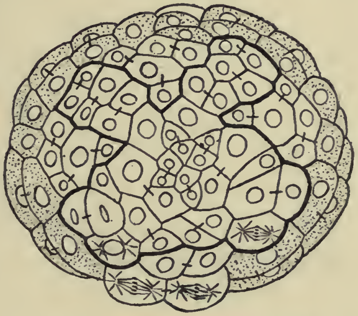

FIG. 21.—*Arenicola.* Descendants of cross cells.

Another good illustration of the lack of correspondence between origin and fate is found in a comparison of the prototroch formation in different forms, *e.g.*, in *Nereis*, *Arenicola*, *Capitella*, *Sternaspis*, *Crepidula*, and *Ischnochiton.* In all these forms except *Sternaspis*, which has no prototroch, the details of the prototroch formation are different, although the cells which form the prototroch arise from the same general region of the egg, and, where the divisions are spiral, of course by similar divisions. In *Sternaspis*, cells corresponding to the so-called primary trochoblasts in *Amphitrite*, etc., are formed, but are simply a portion of the ectoderm. Again, in *Nereis* and *Capitella* the stomatoblasts ("œsophagoblasts") are members of the second quartette, while in *Arenicola* they belong to the third, and in *Ischnochiton* to both second and third. The difference in position of larval mesoblast, or its absence in some cases, indicates independent modification. Numerous other examples of the same kind

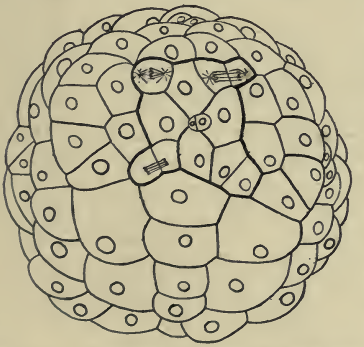

FIG. 22.—*Crepidula.* Upper pole at late stage, showing space occupied by cells corresponding to annelid cross (after Conklin).

might be cited, but these, I think, are amply sufficient for the present purpose.

The facts given above appear to afford conclusive evidence in support of the view that there has been an increasingly early appearance of morphogenetic cleavage and considerable independent modification in the phylogenetic history of the annelids and mollusks and their ancestors. Nevertheless, it must be admitted that there is a high degree of resemblance in the cleavage even of widely separated forms. The tendency has been to emphasize these similarities, even to the extent of neglecting whatever differences might be found. Comparison of details shows that many and considerable differences do exist, and it shows further that the resemblances that occur concern the more general features of the development. Two facts seem to me to point the way to an explanation of these similarities. First, there is in all cases the absolutely identical (or reversed) spiral form of cleavage in the earlier stages to serve as a basis for later modifications. And, second, the larval form, when present, is similar in the different groups. Where the larval form is not present, as in the oligochætes and leeches, the cleavage departs at an early stage from the plan adhered to in the other groups. Whether the trochophore be regarded as palingenetic or cœnogenetic, its similarity of form in the various groups must have been closely connected with the precocious appearance of resemblances in earlier stages of development. Conklin remarks: "The 'reflection' of similar larval or adult characters would produce similar effects upon different eggs, and consequently *the similarity of the prelarval stages of annelids and mollusks may be held to be due to the similarity of their larvæ;* but there is no reason for supposing that this parallel precocity has been independently acquired by annelids and mollusks, since it may well have been produced before the phylogenetic separation of these groups." Conklin has not recognized the influence of the strictly spiral form of cleavage of the early stages in producing similar later stages, probably because he regards the whole cleavage from the beginning as morphogenetic. I agree with him in regarding it as quite possible that the "reflection" or most of it occurred before the separation of the groups, and indeed I think it quite probable of annelids and mollusks. The trochophore, reduced to its simplest terms, doubtless arose from a larval form which occurred in the life history of the common ancestor of the two groups, and which I believe was probably not greatly different from the polyclad larva. But the question as to whether the trochophore is an ontogenetic recapitulation of an ancestral form which was introduced into the ontogeny before the separation of the groups must not be confused with that of its homology within the groups. I do not desire to discuss this matter in detail at this point, but simply to remark that even the high degree of cœnogenetic modification involved in the precocious appearance of morphogenetic cleavage need not necessarily result in a cœnogenetic larval form. I am, however, inclined to regard the original of the trochophore as distinctively a larval form and not as a stage in the phylogeny of the adult forms of the groups in which it belongs.

We have seen above that the spiral period is followed by a distinctly morphogenetic form of cleavage, and that this morphogenetic period has been encroaching upon the earlier spiral period. We find, moreover, that the morphogenetic period shows in large part a distinct bilateral symmetry, and we now turn to a consideration of the significance of this symmetry, *i.e.*, the significance of the direction of cleavage during this period. Notwithstanding some differences, a comparison of various forms shows that the more important bilateral divisions occur with a considerable degree of regularity and in the same cell generation as a rule. Moreover, these are the first evidences of bilaterality. In no case have distinctly bilateral divisions been found at an earlier stage. Wilson regarded this fact as one of the most striking features of the cleavage. In *Crepidula* the change is not so abrupt, according to Conklin, though it appears in almost exactly the same cell generation as in *Nereis*, at least in certain parts of the egg. Wilson believed the appearance of bilaterality was due to the reduction of the "left posterior macromere" to the same size as its fellows as the result of the formation of the first somatoblast and the mesoblast. Conklin has shown, however, that this rule does not hold good in the case of *Crepidula* and many other gasteropods, since here the "left posterior macromere is not appreciably larger than the right, and in some (*e.g.*, *Umbrella*, Heymons, '93) it is smaller, and . . . the mesoblast (4*d*) is only one member of a quartette which is separated in a left spiral from the macromeres, each of the other members being quite as large, or even larger than the cell 4*d*." And finally, after consideration of the manner in which bilateral cleavages arise, he says: "The conclusion, therefore, is unmistakable that bilaterality first appears in processes which lead to the formation of the trunk and the elongation of the future animal, while the primitive radial symmetry of the anterior quadrants is correlated with the fact that these quadrants give rise largely to larval organs, most of which bear traces of radial symmetry." And again it must be said of this conclusion, as Conklin said of Wilson's, that it is not applicable here, or applicable only in modified form, for the anterior quadrants show a high degree of bilaterality in many forms.

I believe the facts as known thus far indicate that the function of bilateral cleavage is *the symmetrical distribution of the material for the formation of the adult or larva.* It is evident that in the adaptation of means to end symmetrical divisions would be useless at a stage when the material was not in a position to be distributed. Suppose the radial divisions forming the cross (Fig. 7) appeared before the cross cells were surrounded by a complete ring of cells. The formation of the cross would not result in forcing the ectodermal cap down over the egg, but would simply produce four lines of cells extending outward from the anterior pole as far as or beyond the other ectoderm cells. Again, suppose the first somatoblast 2*d* were to divide bilaterally at its first division or its second: the growth of the somatic plate in the direction which is to result in its concrescence would not be begun; but, as it is, two cells are formed, lying on either side of, and posterior to, the stem cell (Fig. 9), and then a cell is given off anteriorly in the median line (Fig. 10), and thus the whole mass is forced somewhat posteriorly and the symmetrical distribution of the material is begun. Then in succeeding divisions of the stem cells (Fig. 11–17) how exactly the relation between the parts is preserved, every division balancing others and aiding in the accomplishment of the "desired" end, *viz.*, the concrescence of the plate and the formation of the "growing tip"! Moreover, the posterior movement of the descendants of the first somatoblast which is initiated by the third division — the first symmetrical division — begins the covering over of the mesoblast. The mesoblast divides bilaterally at its first division, for it is formed in such a position that it must be covered over by the somatic plate and thus forced into the interior of the egg, and its fate is to furnish the double "Anlage" for the mesoderm bands. I think these examples are sufficient to show how exactly the appearance of the bilateral cleavages is timed for the accomplishment of the purpose of development in the most perfect manner. And, to my mind at least, they furnish ample explanation of the fact that the bilateral cleavage does not appear in the earlier stages.

It may be urged as an objection to this view that in various forms (*e.g.*, ascidians) the first cleavages are bilateral, and the distribution of the material is accomplished equally well. This, of course, is perfectly true, but there is no evidence that the bilateral form of cleavage has displaced a spiral form in this case. In the annelids and mollusks the spiral form of cleavage is undoubtedly older than the bilateral form, and has been in part displaced by it, the change occurring first in later stages, and proceeding to the earlier. The point made here is simply that there is no adequate cause for the displacement of the spiral by the bilateral form of cleavage in stages earlier than those at which the latter now appears, unless of course the spiral period could be eliminated at one blow, as it were, and a bilateral cleavage could begin at once. This may perhaps be a more complete modification, but there are no indications discernible of its appearance, unless, indeed, the cleavage of such forms as *Teredo* and *Cyclas* is to be regarded as such. The spiral form of cleavage seems to serve perfectly in plotting out the material of the egg in these forms.

In general, the direction of each cell division in morphogenetic cleavage plays a perfectly definite rôle in the accomplishment of the morphogenesis of the species concerned. Bilaterally symmet-

rical cleavage is necessary for the symmetrical distribution of the precociously segregated material, so that it is to be expected that the axes of symmetry in the cleaving egg should correspond with the axes of symmetry in the adult. It is probable, however, that the establishment of these axes is significant as an incident connected with the mechanics of this form of cleavage, rather than as indicating differentiation.

The time of division in the different blastomeres is also a factor in the result attained. As mentioned above, the time at which the spiral form of cleavage ends is closely connected with the process of gastrulation, etc. All through the bilateral period of cleavage the divisions seem to occur at the right time to serve as a factor in morphogenesis. In the case of symmetrical divisions on the two sides of the egg this fact is especially noticeable, and the best illustration is found in the later divisions of the somatic plate (Figs. 11–17). The symmetrical divisions occur at the same time, or nearly, although they may be almost on opposite sides of the egg. Sometimes one side is slightly ahead of the other, but in any case the variation is not great, and the arrangement of the material is the same in every case.

In general, it may be stated that the larger cells divide more rapidly than the smaller, but this rule does not hold good in all cases, and does not apply of course to yolk-laden cells. Indeed, as Conklin ('97) and Lillie ('95) have suggested, the rapidity of division is apparently regulated in some cases by the time at which the portion of material concerned is to become functional, but neither does this rule apply to all cases. For instance, the primary trochoblasts in *Arenicola* are relatively smaller than in *Amphitrite* and *Clymenella* (*cf.* Figs. 7 and 23), and become ciliated at a much later stage. Yet in the two forms the trochoblasts pass through all of their divisions with equal rapidity, *i.e.*, relatively to the other blasto-

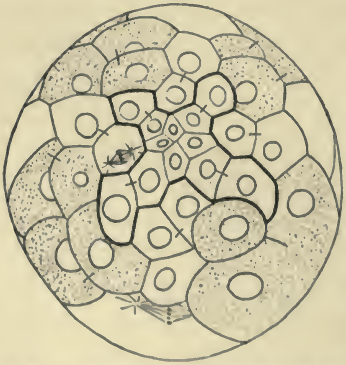

FIG. 23.— *Clymenella.* Small size of cross cells and large size of primary trochoblasts (after Mead).

meres. The early division of these cells in *Arenicola*, where the cells do not become ciliated for a long time, must, I think, be considered as without significance for the prototroch itself, but as directly connected with the overgrowth of the entomeres by the ectomeres. In *Sternaspis* the divisions of the cells which correspond to the trochoblasts occur at almost exactly the same time relatively to the other cells, but here there is no prototroch, and these cells apparently form merely a part of the ectoderm.

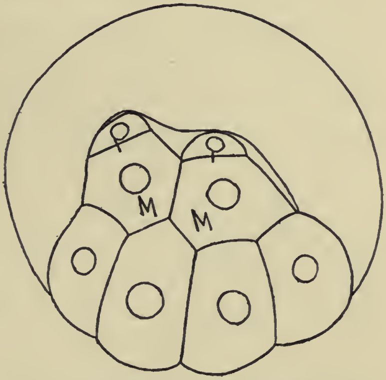

FIG. 24.—*Arenicola*. Mesoblasts *MM* nearly filling the blastocœle. Optical section.

The mesoblast in *Arenicola*, though larger than most other cells in the egg, divides very slowly. This is not wholly because of the fact that it becomes differentiated only at a late stage, for the anterior ends of the mesoblast bands form muscles in the trochophore. But there is no room for the products of its division in the blastocœle in early stages (Fig. 24). If it divided rapidly, invagination of the entomeres would be impossible, unless the ectoderm were stretched to a considerable degree. In cases where the mesoblast is smaller, the rapidity of division may be more closely connected with the time at which it becomes functional as mesoblast.

The size of the various cells is another factor. It is apparently correlated with several features of the process of morphogenesis, *viz.*, the final fate of the cells in question, the stage at which the material is used, and the relation to other cells in the complex. Lillie and Conklin have called attention to the first two of these factors, but the third seems fully as important as either of the other two. The size of the various cells is an essential factor in the accomplishment of the processes of growth. Examination of the growth of the somatic plate gives the impression that not only is the direction of division adapted to the form of growth, but the size of each cell also.

A bit of material is given off at one point, serving to fill up a chink, as it were; a large cell appears at another point, etc., and every cell in the complex aids in the postero-lateral growth and concrescence of the plate, not simply because it was formed in a certain position, but because it is of a certain size. Some cells, like the turret cells of *Crepidula*, are small when formed, but later increase greatly in size. In these cases it seems probable that the appearance of a large cell at the time when these divisions occur would interfere with the distribution of the ectodermal material. The later increase in size may be connected with the rôle which the cell itself is to play, or may be another means of aiding in the distribution of the material.

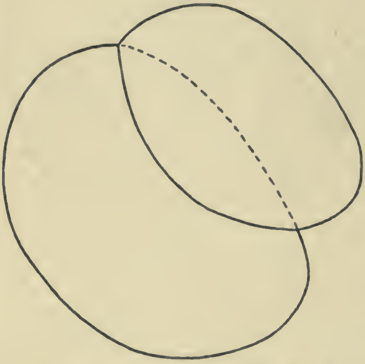

FIG. 25.—*Arenicola.* Unequal two-cell stage from upper pole.

But in many cases a great difference in size is found among the cells at the very beginning of cleavage, as evidenced by the extremely unequal division in many forms (Fig. 25). This difference continues to be expressed at various points during the spiral period, notably in the formation of the first somatoblast, *2d* (Fig. 5), and the mesoblast, *4d*, and, in a less degree, in many other cells. These are the differences which have especially attracted the attention of nearly all those who have studied this form of cleavage. They have been quite generally regarded as differential in significance. There is here undoubtedly an anticipation of, and provision for, the later stages of cleavage, but, to me at least, it seems that, so far as morphogenesis is concerned, it is, at least usually, rather quantitative than qualitative. Certain *amounts*, rather than certain kinds, of material are stored up in certain cells just where they will be in position to produce by coördinated action the "desired result." Thus a still further "condensation" of the process of development is effected. Cells which are to serve as centers of distribution of material, *e.g.*, the first somatoblast, may exceed all other cells of the egg in size, as is the case in *Arenicola*. It seems at least unnecessary to suppose, however, that this cell contains an entirely different "organ-forming substance" from that contained in, for instance, the four cells, products of the dorsal intermediate girdle cell of the first quartette, which pass through the dorsal gap in the prototroch, and lie just in front of the somatic plate (Fig. 13), or from that contained in the cells of the second and third quartettes which form the ectodermal region about the mouth. Indeed, Dr. Treadwell tells me that in *Podarke* a large portion of the dorsal ectoderm of the trochophore is formed by the descendants of the dorsal first quartette cell, which have passed through the gap in the prototroch.

Of course, difference in size between yolk-bearing and non-yolk-bearing cells is an expression of difference in constitution.

Notwithstanding the fact that the spiral form of cleavage remains throughout the earlier stages of development, a form of modification does occur which in a way anticipates the morphogenetic character of the later cleavage. The determinate character of the morphogenetic period establishes the fate of each cell under normal conditions; and, moreover, it renders possible a further degree of modification in the cleavage, *viz.*, "precocious segregation." As the result of precocious segregation, the blastomeres not only have a definite fate, but each contains a definite amount of material, according to the part which it is to play in the cleavage and irrespective of the yolk which it may contain. This modification extends back to the earliest stages of cleavage, but without altering the spiral form, notwithstanding the great differences in pressure that may exist between the blastomeres. It has become the custom to speak of a protoblast as containing from its earliest appearance the substance for the organs which are to be formed by its descendants, and the term "differential cleavage" has been employed to denote the divisions which give rise to the protoblasts. Precocious segregation is undoubtedly connected with differentiation in so far as the perfectly exact distribution of the material renders an earlier differentiation possible; but, as regards the separation of various organ-forming substances by the planes of cleavage, it is very difficult for me, at least, to believe that it occurs, especially as I cannot see that there is evidence to warrant the conclusion. There are, of course, cases where certain substances have been found to pass always into definite cells, *e.g.*, the granules of the "Urvelarzellen" of *Neritina* (Blochmann, **'81**) and certain granules in *Asplanchna* (Jennings, **'96**). Evidence is lacking, however, to show that these substances are necessary to the formation of the organs to which the cells give rise. Conklin (**'99**, p. 18) admits that there is abundant evidence to prove the absence of any necessary relation between cell formation and differentiation, but believes that in certain cases the two processes are related. He distinguishes three categories of relation: (1) "Cell formation following the lines of preceding differentiation, *e.g.*, certain cleavages of ctenophores, mollusks, and ascidians; or (2) cell formation and concomitant differentiation, *e.g.*, many cleavages of turbellarians, nematodes, annelids, and mollusks; or (3) differentiation following the lines of preceding cell formation, *e.g.*, many cleavages in the eggs of annelids, mollusks, and probably many other animals." The significance of these so-called relations becomes apparent when we observe that every differentiation which occurs in multicellular material must be related in one of these three ways to cell division. In other words, cell division and differentiation are just as independent of each other here as elsewhere, but the process of differentiation is occurring to a certain extent during a series of very conspicuous cell divisions, *i.e.*, the earlier ones. A comparison of the process of formation of a single organ, the prototroch, in different animals seems to me to show how absolutely distinct cell formation and differentiation are. The prototroch may consist of eight or of sixteen cells, of twenty-five, of thirty, or of many; the series of divisions leading to its formation are almost always different in different forms, and where they are not they are similar simply because they are purely spiral cleavages; it may become functional almost immediately after the divisions are completed, — indeed in *Capitella* (Eisig, **'98**) divisions continue after ciliation, — or it may remain a long time before becoming ciliated; and, finally, cells perfectly equivalent to the "primary trochoblasts" in origin may be formed without ever forming a prototroch.

It is hardly necessary to remark that the differentiation of an egg into protoplasmic and deutoplasmic portions, which so often accompanies cleavage, undoubtedly has, in most cases at least, a comparatively simple explanation.

Differentiation is then, I believe, "a function of position" (*cf.* Driesch). *Whether the material is contained within one cell or many is a mere incident so far as the result is concerned. The comparative study of the spiral type of cleavage leads irresistibly to this conclusion.*

The appearance of definite protoblasts in cleavage does not necessarily imply that they contain a specific material which is necessary for the formation of the organ in question. Protoblasts are, I think, to be regarded, in general, as centers of distribution of the material of the egg; and their formation is probably due to a condensation in the process of development, or a saving of energy, as Conklin suggested. As I understand Conklin's position, however, he regards the saving of energy as occurring in processes of differentiation merely, for, as he remarks, "it is possible to see that in an organ which reaches functional activity after a dozen divisions less energy has been expended than in one which reaches this stage only after one hundred divisions." This is undoubtedly true, but how does it explain the formation of the whole trunk ectoderm from the first somatoblast, which occurs in some annelids, or the formation of the mesoblasts, for the descendants of these cells do not, for the most part, become "functional" in the definitive sense for a long time, and then chiefly with reference to the adult body and not the larval? There is surely no reason for believing that the number of cells in the organs of the adult is less in those forms in which precocious segregation has occurred than in others. Lillie ('95) has pointed out the fact that "almost every detail of the cleavage of the ovum of *Unio* can be shown to possess some differential significance." It is undoubtedly significant as regards the distribution of the egg material, but that is not necessarily differentiation. The material separated as the result of precocious segregation may, I believe, be perfectly indifferent material, except as regards position, and the true significance of the segregation may lie in the fact that a certain *amount* of material is present, which is to be distributed through the means of a series of perfectly definite cell divisions. By "distribution" is meant the arrangement of the material in such a manner that the various morphogenetic processes are directly accomplished thereby, *e.g.*, formation of the somatic plate, gastrulation, which in *Arenicola* and some related forms is probably accomplished very largely by the growth of the somatic plate, formation of the mesoderm bands, beginning elongation of the larva, etc. The important point in this view lies, I believe, in the conclusion that these results are accomplished, at least very largely, directly by the energy of cell division itself. Given a certain degree of cohesive power in the protoplasmic material, a certain degree of surface tension, and a certain definite series of cell divisions and the result must always be the same, and the same cells will always occupy the same relative positions at corresponding stages and will form the same parts of the whole, *i.e.*, will have the same fate under normal conditions. This would be true even if the segregated material should remain totipotent long after its segregation.

Since the fate of the various cells is definitely established and can be foretold, it is possible that differentiation might begin at an earlier stage than otherwise, but there is no reason to suppose that the process of cell division is connected with it even here. To take an example: why should the two lateral cells of the paratroch on each side in *Arenicola* pass through one more division than the dorsal cells before they form the paratroch (Fig. 15)? If cell division is connected in these eggs with differentiation, it must be either that one of these divisions is "non-differential," according to Conklin's distinction, or else it is necessary to assume that the lateral cells are more highly differentiated than the dorsal cells. As regards the first assumption, there seems to be no definite criterion of differential divisions, so that no decision is possible. As regards the second, there is no reason to believe that it is true. Taking up the matter from a different point of view, *viz.*, the relation of the constituent parts with regard to the result to be accomplished, an explanation appears possible. This division is a necessary step in the concrescence of the somatic plate. It does not bring "paratrochal material" into the proper position or into the proper degree of differentiation, but it does aid in accomplishing the end toward which each of the symmetrical divisions in the somatic plate contributes its part. In another case, *e.g.*, *Amphitrite*, where some of the preceding divisions are different and the material is somewhat differently grouped, no such division occurs, and the paratroch consists of four cells instead of six.

The extreme precocious segregation, which extends back even to the first cleavage and in some forms (*Arenicola*) is indicated even before cleavage by the shape of the egg (Figs. 26, 27), is rendered possible by the fact that the whole cleavage is strictly determinate in character. A determinate form of cleavage is, as I believe, the product of a considerable degree of modification in development in the direction of condensation. Thus the appearance of precocious segregation in the earliest stages must indicate a still further departure from the original primitive forms of cleavage.

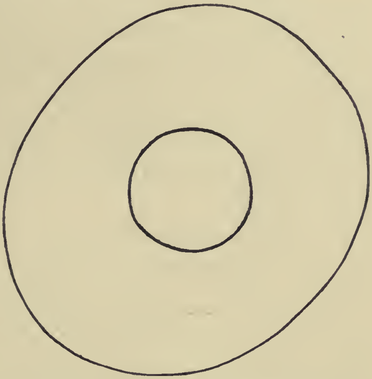

FIG. 26.—*Arenicola*. Egg before division, seen from upper pole.

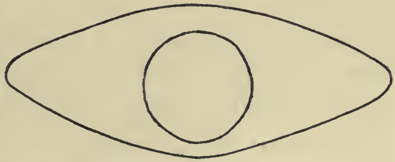

FIG. 27.—*Arenicola*. Egg before division, seen from side.

Precocious segregation reaches its highest expression at present in the oligochætes and the leeches, especially in the latter, where the development is very largely teloblastic, the material for almost the whole body being segregated into a few cells which form the germ bands. Here is not only segregation, but regulated cell division in the highest degree.

Teloblastic development must undoubtedly be regarded as the last term in a long series of modifications, all leading to a shortening or condensation of the process of development, although this, as Conklin has remarked, does not necessarily signify a shortening of the time of development. I have attempted in the preceding pages to follow the various steps of the process, as I believe they must have occurred historically.

It appears probable that a large amount of yolk affords a condition favorable to the teloblastic form of development, for it renders possible the very early localization of the embryo upon certain portions of the egg. In the annelids and mollusks the amount of yolk in the egg is not sufficient to render any such localization of material necessary in the interests of condensation of development, and a partial localization of material at various points is the most favorable arrangement for rapid development.

In any form of cleavage which is morphogenetic, it is incorrect to regard certain cells as becoming functional at certain times. Each cell is functional at every stage of the cleavage and directly connected with morphogenesis. Since this is the case, it is necessary in seeking for the explanation of its various qualities, such as structure, size, direction, and time of division, etc., to consider, not simply the part which it is to play in the adult organism or in the larva, but the part which it plays at the moment under consideration and at each moment before and after it. Each stage of development is the result of the preceding stage and in some manner the cause — not of the adult or larval organization — but simply of the next succeeding stage. Each stage is a whole, not a series of independent parts, and, moreover, modifications may occur in any stage which are not connected — except, of course, indirectly, since development is continuous — with the final organization, but relate only to some detail of some particular stage. Thus we may expect to find numerous differences in detail in the solution of the same general problem, *i.e.*, the production of the same general organization.

I believe that, without an adequate appreciation of the extreme plasticity of the cell, a true conception of organization is impossible. The cell may be likened to a bit of plastic material, which may be modeled according to the needs of the organism, and which, even when once modeled, may be brought back, like a bit of clay, to the original condition and remodeled into a different form if necessary. And further, the material connection between cell and cell, which occurs so widely, is of the utmost importance, for through this, as I believe, the cell becomes simply a part of the organism, in touch with the whole, influenced by the whole, and exercising in its turn an influence upon the whole. Whether that peculiar property of protoplasm which we know as organization be merely the expression of its chemical and physical structure, or, as Driesch believes ('**99**), the expression of a mysterious force wholly different from any in the inorganic world, it is the *organism — the individual, which is the unit and not the cell.*

University of Chicago.

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FIFTEENTH LECTURE.

THE AIMS OF THE QUANTITATIVE STUDY OF VARIATION.

C. B. DAVENPORT.

THERE are two fields of investigation possessed of well-defined methods whose rise and development have characterized the advance of zoölogical morphology in the present decade. The first of these is experimental morphology, or (to use a more restricted term) experimental embryology. Experimentation is not new, even in morphology, but there has been a decided advance upon the methods in vogue a century ago. The second new field of investigation is the precise, quantitative study of variation. The method is here also in part old, having long been used in anthropology; but in its application to zoölogy in the strict sense it is quite new. I wish to point out what are the aims of this study.

The aim of quantitative variation study may be stated generally to be the investigation of the specific[1] form, including the laws of its development and maintenance in the individual, the laws of intermingling of specific forms in the progeny of parents having dissimilar specific forms; and the laws of evolution of new specific forms in nature; *i.e.*, the method of origin of species. I will treat of these three subdivisions of the field of quantitative investigation in inverse order.

In studying specific differentiation in nature we shall find it, first of all, necessary to investigate species as we find them in

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[1] "Specific" is here used in a broad sense to cover the form-peculiarities of families, communities, races, species, genera, in short, peculiarities constant to a group of related individuals of whatever extent.

nature; not indeed the species of the systematist, but rather those homogeneous lots of individuals taken from one locality to which Duncker has given the convenient name "form-units." The quantitative method of studying the form-unit consists in counting certain numerically repeated organs, or of measuring one or more organs, on each of many individuals. The quantities resulting from these measurements are then "seriated" or arranged in classes according to size. More quantities will fall in certain of these classes — usually the middle classes — than in the others. The distribution of "frequencies of occurrence" in the several classes will give those characters of the form-unit for which we are seeking — its rigidity or plasticity, its stability or instability, its independence of or subjection to selection, and its unity or its tendency to break up into several forms. From a comparison of measurements of two or more organs of the same individuals we may learn additional facts concerning the form-unit; such as the degree of correlation existing between its different parts, from which we may infer directly the morphogenetic kinship of the different organs, and, more indirectly, get light on homologies. The quantitative study of the form-unit thus bears on many biological topics.

The possibility of getting such biological data from statistics of organisms depends upon the fact that biological statistics — more particularly the distributions of "frequencies" — follow definite laws capable of mathematical expression and analysis. The mathematical analysis necessary to deduce the biological results is not very difficult, but is quite within the range of any one who has studied trigonometry and logarithms.[1]

The objection has been raised that the quantitative method of investigating the form-unit is limited to organs which can be counted or measured with a millimeter scale. It is, however, applicable to the study of all quantities which can be

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[1] For an account of the methods of dealing with biological statistics, see a paper by Dr. Georg Duncker, on "Variations-Statistik," in Roux's *Arch. f. Entwickelungsmechanik*, 1899, sold separately by W. Engelmann, Leipzig; and my little book, Statistical Methods with Special Reference to Biological Variation, published by John Wiley & Sons, New York, which contains also all the tables necessary for the mathematical analysis of frequency distribution.

measured; practically to all qualities of form and color which an organism possesses. The thorough application of the quantitative method often takes time, practice, and ingenuity, but the results are worth the cost.

As stated above, the distribution of frequencies in the several classes gives a measure of the stability or variability of an organ. Let us consider what this single result enables us to do. It enables us to express the laws of normal variation quantitatively instead of loosely, as heretofore. Thus we can now compare the relative variability of the sexes; we can determine whether wide-ranging species are especially variable; whether specific characters are more variable than generic ones; whether parts developed to an extraordinary degree are especially variable; whether in serially repeated organs those standing at the ends of the series vary most; and whether secondary sexual characters are more unstable than other somatic ones. All these and other "laws" can be established only by quantitative methods.

Again, in systematic zoölogy this method will be of great use. I know that systematic zoölogy is much decried in some schools, and unquestionably it has suffered from the adherence of some unphilosophic workers. On the other hand, many of the workers in this department of zoölogy have been brought face to face with the great problem of the origin of species, have seen their opportunity, and have contributed valuable data. If they have failed to do more, it is because the methods they have used — the methods of adjective descriptions — have been wholly inadequate to the task. What headway can one make on the classification of the American Unios by saying that the individuals from one river differ from those of another by being more elongated, or rounder, or browner, or by having a lower beak, or sharper radiating lines. Not until these qualities and others are reduced to numbers can we hope to disentangle this maze of varying communities. After we shall have a number of studies made in this fashion upon wide-ranging species or genera, we shall have gained some adequate notion of the relation of the origin of species to geographic distribution.

It has been urged by some naturalists that the quantitative study of animals will take away that peculiar charm which attaches to biological study. This peculiar charm, as I understand it, is due to the contact with out-of-doors, into which the biologist is brought, and to the fact that the phenomena which he studies of growth, reproduction, variation, inheritance, and differentiation of form and activities, of structure and habits, form an important part of human experience. Now the student of variation also is concerned with these same phenomena, only he uses a different method in his study of them. Moreover, the necessity of collecting large numbers of individuals for study will be especially apt to take the student to the field. After all, it is the search for truth, particularly a truth which seems worth while, which inspires us, and the quantitative method gives us a new tool in this search.

There seems to be an impression that all this quantitative work is very difficult. But this is not at all the case. Many important pieces of work require only abundant material and the ability to count and record. Thus, by counting the ray flowers of the field daisy, Ludwig discovered that in the distribution of their frequencies they exhibit several "modes" occurring in a mathematical series — the series of Fibonicci. By counting the grooves in scallop shells from two Long Island localities, I have found a significant difference in the variability of the upper and lower valves, and also a difference in the average number of grooves from the two localities. With the aid of a hand lens only, a series of studies has been made on the variability of the rostral teeth of prawns from different countries and from the same country, but subjected to various conditions. Of course, with the aid of a millimeter scale, dividers, planimeter, map measurer, color top, balances, and other apparatus, one can go farther. As to the number of individuals required, at least from two hundred to five hundred should be employed when possible.

Not only the qualities of the form-unit, but the causes which have determined those qualities are to be investigated quantitatively. Among these, selection, the direct action of environment, and internal tendencies to change are reckoned the most important. Now how can we know anything definite concerning the causes of specific change unless we can express exactly the present condition of a form-unit and express exactly again its condition after the working of a factor? Descriptions based on adjectives are inadequate for a precise expression either of the present condition of a character or of a slight change in it. The beautiful results on the change in form of the crabs at Plymouth, which Weldon obtained by the quantitative method, could hardly have been obtained without that method. It has long been a reproach to biology that it could give so few demonstrations of race differentiation going on in nature. It has, indeed, been pointed out that we should not expect in man's brief lifetime, nor even within historic times, to find such changes; but this defense is valid only if we rely upon adjectives for specific descriptions. If specific descriptions are given quantitatively, on the other hand, we can hope soon to prove that many species are undergoing change and to determine the cause of the change.

Again, correlation is an important biological conception; it is especially important for the determination of homologies, for homologous organs exhibit, as is well known, a tendency to vary correlatively, *e.g.*, the right and left sides of the body; the series of teeth or ribs. Accordingly within certain limits one can infer homology from correlation. A precise measure of correlation consequently will serve as a measure of morphological kinship. Such a precise measure is given by "Galton's function."

Intimately associated with the study of correlation between organs of the body is the quantitative study of heredity, or the correlation between parent and offspring or between other related individuals. In this field there are numerous matters demanding thorough investigations. There is the confirmation of Galton's law of Ancestral Inheritance, by which all the parents of any (nth) degree of ancestry (the parents being of first degree, grandparents of second, etc.) contribute together $\frac{1}{2^n}$ of the total heritage. There is the matter of prepotency in breeding — prepotency of one race or one sex. Prepotency is the preponderating transmission from one parent. The very definition implies a quantitative difference in the power of transmission. A great difference may indeed be detected and expressed without resorting to measurements ; but it will often happen that there is true prepotency even if slight, and such prepotency must go unobserved without the use of the quantitative method. Then there is the problem, so important for the question of species, of the relative potency of sports and the normal form when mated. Even a slight prepotency, too small to be detected qualitatively, may suffice to prevent the sport from being swamped by crossing with the normal form. Again, there is the question of telegony. Whether the first sire influences subsequent offspring can be affirmed or denied only by comparing the correlation between offspring and previously cross-mated mother with the correlation between offspring and previously pure-mated mother. If there is less correlation in the first case than in the second, telegony exists. The facts of xenia are to be investigated in the same way. Still further, the phenomena of blending of parental characters in hybridization, or of their alternative heritage, or their patchwork intermingling — all these phenomena demand careful quantitative study.

Finally, even the development of the specific form in the individual demands quantitative investigation. Many investigators have already studied embryonic variation, but few quantitatively. The precise comparison of variability in the embryo and the adult will be of importance on account of its bearing on theories of selection and self-adaptation. The most important results in this field are, however, clearly gained by the quantitative study of variation in embryos subjected to varying environmental conditions. The exact comparison of change of environment with change in form will lead to a still more precise knowledge of the effect of external agents. So likewise the quantitative studies made on the result of experiments to produce race change will be the most striking. In fact, in the application of combined experimental and statistical methods to genetic problems, zoölogy will reach its highest development.

SIXTEENTH LECTURE.

ON THE NATURE OF THE PROCESS OF FERTILIZATION.

JACQUES LOEB.

I.

LEEUWENHOOK demonstrated in 1677 the existence of spermatozoa. It was about one hundred and sixty years before biologists convinced themselves that these spermatozoa were no parasites. In 1835 K. E. von Baer was still of the opinion that the spermatozoa had nothing to do with the process of fertilization. The parasitic conception of spermatozoa was finally done away with by Wagner's demonstration that only those animals are capable of fertilizing eggs whose sperm contains spermatozoa. Very soon afterwards histologists showed the origin of spermatozoa from cells.

Leeuwenhook was of the opinion that the spermatozoön represents the future embryo. On the other hand, it was not difficult to notice that the embryo in fishes, amphibians, and birds develops from an egg furnished by the female. The question arose as to what was the homologous element in the female of mammals. In 1672 De Graaf discovered the follicle in the ovary of mammals, and in 1827 von Baer discovered the mammalian egg cell.

The next problem that was solved in this branch of science was the relation of the sperm to the egg. Many scientists, among them De Graaf, had assumed that no direct contact between egg and sperm was necessary, that something volatile in the sperm, the aura seminalis, was sufficient for the act of fertilization. Contrary to this, Jacobi showed (1764) that fish eggs can only be fertilized by bringing the sperm into direct contact with the eggs, and Spallanzani showed the same for the frog. He succeeded in producing artificial fertilization of mammals by introducing sperm into the vagina. But even Spallanzani did not realize that the spermatozoa were the essential element in the sperm. In 1824 Prévost and Dumas proved this by filtering the sperm, and demonstrated that the sperm whose spermatozoa had been retained by the filter lost its power of impregnating the egg. These observations established the fact that the spermatozoön has to come in contact with the egg in order to bring about fertilization.

The next step was the observation made by Barry in 1843 that the spermatozoön actually enters into the egg. This observation was confirmed ten years later by a number of authors, Meissner, Newport, Bischof, etc. It is rather remarkable that it was one hundred and sixty years after the discovery of the spermatozoön and the follicle before the fact was recognized that the spermatozoön has to enter the egg in order to bring about fertilization. Had the biologists during these one hundred and sixty years lost their interest in the investigation of this problem ? This was certainly not the case, but they spent their energy not in fruitful research, but in speculations and controversies which were admired by their contemporaries and made their authors famous, but which were a mere waste of time. History has since repeated itself in other fields of biology. The outcome of the facts gathered concerning the process of fertilization was four apparently different theories of fertilization, which, however, have much in common.

The first theory of fertilization is a morphological one. According to this theory, it is the morphological structure of the spermatozoön which is responsible for the process of fertilization.

The second theory is a chemical one. According to this theory it is not a definite morphological or structural element of the spermatozoön, but a chemical constituent, that causes the development of the egg. Against this second view Miescher has raised the objection that his investigations showed the same compounds in the egg and the spermatozoa. I do not think that this objection is valid. We know that simple variations in the configuration of a molecule have an enormous effect upon life phenomena. This is shown among others by the work of Emil Fischer on the relation between the molecular configuration of sugars and their fermentability. When Miescher made his experiments he was not familiar with such possibilities. Moreover, Miescher was not able to state whether the spermatozoa contain enzymes or not.

A third theory was a physical theory (Bischof). This theory assumes that a peculiar condition of motion exists in the spermatozoön which is transmitted to the egg and causes its development. It should be said, however, that this idea is not so very different from the chemical conception, because it assumes exactly the same for the spermatozoön that Liebig assumes for the enzymes. Liebig thought that the enzymes owed their power of producing fermentation to the motions of certain atoms or groups of atoms.

The fourth conception is the stimulus conception, which was originated by His. According to this conception the egg is considered as a definite machine which if once wound up will do its work in a certain direction. The spermatozoön is the stimulus which causes the egg to undergo its development. It is to be said in connection with this stimulus conception that the main point at issue is omitted, as to whether the stimulus carried by the spermatozoön is of a physical or a chemical character, and in this way, of course, the stimulus conception is nothing but a disguised repetition of the chemical or physical theory of fertilization.

All these theories are so vague that we do not need to be surprised that none of them has led to any further discovery. If we want to make new discoveries in biology, we must start from definite facts and observations, and not from vague speculations. Among these observations the most important are those on parthenogenesis. It had been observed for a long time that the unfertilized egg of the silkworm can develop parthenogenetically. It was, moreover, known that plant lice can give rise to new generations without fertilization. The most impressive fact concerning the parthenogenesis of animals was contributed by Dzierzon, who discovered that the unfertilized eggs of bees develop and give rise to males, while the fertilized eggs give rise to females. Similar conditions seem to exist in wasps. It is, moreover, certain that a few crustaceans show parthenogenesis.

A beginning of parthenogenetic development had been observed in the case of a great many marine animals which develop outside of the female in sea water. It was found that such eggs when left long enough in sea water may develop into two or three cells, but no further. (2) On the other hand, in ovaries of mammals now and then eggs were found that were segmented into a small number of cells. These facts and the occurrence of a certain class of tumors in the ovary, the so-called teratomata, suggest the possibility of at least partial parthenogenesis in the eggs of mammals. But all these phenomena were considered to be of a pathological character. It must be, however, admitted that we cannot utilize these facts with any degree of certainty for the theory of fertilization, as in this case certainty can only be obtained by the experiment. It was not until very recently that such experiments were made.

II.

Eight years ago I observed that if the fertilized eggs of the sea urchin were put into sea water whose concentration was raised by the addition of some neutral salt they were not able to segment, but that the same eggs, when put back after they had been in such sea water for about two hours, broke up into a large number of cells at once instead of dividing successively into two, four, eight, sixteen cells, etc. Of course it is necessary for this experiment that the right increase in the concentration of the sea water be selected. (3) The explanation of this fact is as follows : The concentrated sea water brings about a change in the condition of the nucleus which permits a division and a scattering of the chromosomes in the egg. As soon as the egg is put back into normal sea water it breaks up into as many cleavage cells at once as nuclei or distinct chromatin masses had been preformed in the egg. Morgan tried the same experiment on the unfertilized eggs of the sea urchin, and found that the unfertilized egg, if treated for several hours with concentrated sea water, was able to show the beginning of a segmentation when put back into normal sea water. A small number of eggs divided into two or four cells, and, in a few cases, went as far as about sixty cells, but no larva ever developed from these eggs. (4) Morgan had used the same concentration of sea water as Norman (5) and I had used in our previous experiments. I had added about 2 grams of sodium-chloride to 100 c.c. of sea water. Norman used instead of this 3½ grams of $MgCl_2$ to 100 c.c. of sea water, and Morgan used the same concentration. Mead made an observation somewhat similar to Morgan's upon Chætopterus. He found that by adding a very small amount of KCl to sea water he could force the unfertilized eggs of Chætopterus to throw out their polar bodies. The substitution of a little NaCl for KCl did not have the same effect. (6) While continuing my studies on the effects of salts upon life phenomena, I was led to the fact that the peculiar actions of the protoplasm are influenced to a great extent by the ions contained in the solutions which surround the cells. As is well known, if we have a salt in solution, say sodium-chloride, we have not only NaCl molecules in solution, but a certain number of NaCl molecules are split up into Na ions ($Na^{\cdot}$ atoms charged with a certain quantity of positive electricity) and Cl ions (Cl atoms charged with the same amount of negative electricity). When an egg is in sea water, the various ions enter it in proportions determined by their osmotic pressure and the permeability of the protoplasm. It is probable that some of these ions are able to combine with the proteids of the protoplasm. At any rate, the physical qualities of the proteids of the protoplasm (their state of matter and power of binding water) are determined by the relative proportions of the various ions present in the protoplasm or in combination with the proteids. (7) By changing the relative proportions of these ions we change the physiological properties of the protoplasm, and thus are able to impart properties to a tissue which it does not possess ordinarily. I have found, for instance, that by changing the amount of sodium and calcium ions contained in the muscles of the skeleton we can make them contract rhythmically like the heart. It is only necessary to increase the number of sodium ions in the muscle or to reduce the number of calcium ions or both simultaneously.[1] On the basis of this and similar observations I thought that by changing the constitution of the sea water it might be possible to cause the eggs not only to show a beginning of development but to develop into living larvæ, which were in every way similar to those produced by the fertilized egg.

There seemed to be three ways in which this might be accomplished. The first way was a simple change in the constitution of the sea water without increasing its osmotic pressure. The second way was to increase the osmotic pressure of the sea water by adding a certain amount of a certain salt. The third way was by combining both of these methods. The first way did not lead to the result I desired. All the various artificial solutions I prepared had only the one effect of causing the unfertilized egg to divide into a few cells, but I was not able to produce a blastula. I next tried the effects of an increase in the sea water by adding a certain amount of magnesium-chloride. In this case I had no better results than Morgan. Very few eggs began to divide, but these did not develop beyond the first stages of segmentation. I then tried the combination of both methods. The osmotic pressure of ordinary sea water is roughly estimated to be the same as that of a $\frac{5}{8}$ $nNaCl$ solution or a $\frac{10}{8}$ $nMgCl_2$ solution. I found, after a number of experiments, that by putting the unfertilized eggs of the sea urchin into a solution of 60 c.c. of $\frac{20}{8}$ $nMgCl_2$ solution and 40 c.c. of sea water for two hours the eggs began to develop when put back into normal sea water. Such eggs reached the blastula stage. I do not think that anybody has ever seen before such blastulæ as resulted from these unfertilized eggs. As these eggs had no membrane, the amœboid motions of the cleavage cells led very frequently to a disconnection of the various parts of one and the same egg, and the outlines of the egg became extremely irregular. The blastulæ

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[1] It is due to the Ca ions of our blood that the muscles of our skeleton do not beat rhythmically like our heart.

showed, as a rule, the same outline as the egg had in the morula stage. It was, moreover, a rare thing that the whole mass of the egg developed into one blastula. The disconnection of the various cleavage cells led, as a rule, to the formation of more than one embryo from one egg. The results were in a certain way similar to those I had obtained when I caused the fertilized eggs of sea urchins to burst. In such cases a part of the protoplasm flowed out from the egg but was able to develop. These extraovates had no membrane, and of course showed some irregularity in their outlines, but the irregularity in this case was far less than that observed in the unfertilized eggs of my recent experiments. But although I had thus far satisfied my desire to see the unfertilized eggs of the sea urchin reach the blastula stage, I was not able to keep these eggs alive long enough to see them grow into the pluteus stage. They developed more slowly than the normal eggs, and died, as a rule, on the second day.

It was my next task to find a solution which would allow the eggs to reach the pluteus stage. I found that this can be done by reducing the amount of magnesium-chloride and increasing the amount of sea water. By putting the unfertilized eggs for about two hours into a mixture of equal parts of $\frac{20}{8}$ n$MgCl_2$ and sea water, the eggs, after they were put back into normal sea water not only reached the blastula stage, but went into the gastrula and pluteus stages. The blastulæ that originated from these eggs looked much healthier and more normal than those of the former solution with more $MgCl_2$. Of course as these unfertilized eggs had no membrane it happened but rarely that the whole mass of an egg developed into one single embryo. Quadruplets, triplets, and twins were much more frequently produced than a single embryo. The outlines of each blastula were much more spherical than in the previous experiment. These eggs reached the pluteus stage on the second day (considerably later than the fertilized eggs do). Thus I had succeeded in raising the unfertilized eggs of sea urchins to the same stage to which the fertilized eggs can be raised in the aquarium. I have not yet succeeded in raising the fertilized eggs in my laboratory dishes beyond the pluteus stage.

Though I do not wish to go into the technicalities of these experiments, I must mention a few of the precautions that I took in order to guard against the possible presence of spermatozoa in the sea water. The reader who is interested in this technical side of the experiments will find all the necessary data in my publication in the *American Journal of Physiology* (8). Here I only wish to mention the following points: —

1. These experiments were made after the spawning season was practically over. 2. Bacteriological precautions were taken against the possibility of contamination of the hands, dishes, or instruments with spermatozoa. 3. The spermatozoa contained in the sea water lose, according to the investigation of Gemmill (9), their fertilizing power inside of five hours if distributed in large quantities of sea water.

4. We have a criterion by which we can tell whether the egg is fertilized or not in the production of a membrane. The fertilized egg forms a membrane and the unfertilized egg has no distinct membrane. None of the unfertilized eggs that developed artificially had a membrane.

5. With each experiment a number of control experiments were made. Part of the unfertilized eggs were put into the same normal sea water that was used for the eggs that did develop. None of these eggs that remained in normal sea water formed a membrane or showed any development, except that a few of them were divided into two cells after about twenty-four hours. 6. I made another set of control experiments by putting a lot of eggs of the same female into a solution which differed less from the normal sea water than the one which caused the formation of blastulæ or plutei from the unfertilized eggs. In this case it was shown, that although these eggs received the same sea water as the ones which developed, and although they were injured less than the ones which developed, yet not one single egg formed a membrane or reached the blastula stage. If the sea water had contained any spermatozoa these eggs should have reached the blastula stage.[1]

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[1] Through other control experiments I convinced myself that a treatment of eggs or spermatozoa with equal parts of a $\frac{20}{8}$ n$MgCl_2$ solution and sea water diminishes the impregnability of the eggs and annihilates the fertilizing power of spermatozoa in a very short time.

Hence, as in nine different series of experiments these results were confirmed, we may assume that by treating the eggs for two hours with a solution of equal parts of a $\frac{20}{8}$ $nMgCl_2$ solution and sea water we can cause them to develop parthenogenetically into plutei.

III.

What conclusions may we draw from these results? If we wish to avoid wild and sterile speculations, I think we should confine ourselves to the following question: What alterations can be produced in an egg by treating the same for two hours with a solution of equal parts of $\frac{20}{8}$ $nMgCl_2$ and of sea water? Even in this regard we can only give a very indefinite answer, which, however, will have to be in the following direction: The bulk of our protoplasm consists of colloidal substances. This material easily changes its state of matter and its power of binding water. It seems probable that changes of these two qualities are mainly responsible for muscular contraction and perhaps amœboid motions. Among the agencies that cause changes of these physical qualities we know of three that are especially powerful. The one is specific enzymes (trypsine, plasmase, etc.). The second is ions in definite concentration. The concentration varies for various ions. The third agency is temperature. In our experiments it is obvious that only the second possibility can have been active. I do not consider it advisable to enter into theoretical discussions beyond these statements. The next question that should be raised would be whether the spermatozoa act in the same way. It is true that the spermatozoön contains a considerable proportion of salts, especially K_3PO_4, but it may contain enzymes or it may contain substances which have similar effects upon the physical qualities of the colloids, like the three agencies mentioned above.

In the last volume of these lectures I pointed out that it is impossible to derive all the various elements that constitute heredity from one and the same condition of the egg. (10) Our recent experiments suggest the possibility that different constituents of the egg are responsible for the process of fertilization and for the transmission of the hereditary qualities of the male. While we are able to produce the process of fertilization by a treatment of the unfertilized egg with certain salts in certain concentrations, we cannot hope to bring about the transmission of the hereditary qualities of the male by any such treatment. Hence, the inference must be that the transmission of the hereditary qualities of the male and the agency that causes the process of fertilization are not necessarily one and the same thing. I consider the chief value of the experiments on artificial parthenogenesis to be the fact that they transfer the problem of fertilization from the realm of morphology into the realm of physical chemistry.

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